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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12Q 1/00 — A1

(11) International Publication Number: **WO 00/20626**

(43) International Publication Date: 13 April 2000 (13.04.00)

(21) International Application Number: PCT/US99/23425

(22) International Filing Date: 8 October 1999 (08.10.99)

(30) Priority Data:

60/103,627	8 October 1998 (08.10.98)	US
60/105,773	8 October 1998 (08.10.98)	US
09/295,962	21 April 1999 (21.04.99)	US

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(81) Designated States: AE, AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), DM, EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: SMALL VOLUME *IN VITRO* ANALYTE SENSOR WITH DIFFUSIBLE OR NON-LEACHABLE REDOX MEDIATOR

(57) Abstract

A sensor utilizing a non-leachable or diffusible redox mediator is described. The sensor includes a sample chamber to hold a sample in electrolytic contact with a working electrode, and in at least some instances, the sensor also contains a non-leachable or a diffusible second electron transfer agent. The sensor and/or the methods used produce a sensor signal in response to the analyte that can be distinguished from a background signal caused by the mediator. The invention can be used to determine the concentration of a biomolecule, such as glucose or lactate, in a biological fluid, such as blood or serum, using techniques such as coulometry, amperometry, and potentiometry. An enzyme capable of catalyzing the electrooxidation or electroreduction of the biomolecule is typically provided as a second electron transfer agent.

Small Volume *in vitro* Analyte Sensor with Diffusible or Non-Leachable Redox Mediator

Field of the Invention

This invention relates to analytical sensors for the detection of bioanalytes in a small volume sample.

Background of the Invention

Analytical sensors are useful in chemistry and medicine to determine the presence and concentration of a biological analyte. Such sensors are needed, for example, to monitor glucose in diabetic patients and lactate during critical care events.

Currently available technology measures bioanalytes in relatively large sample volumes, e.g., generally requiring 3 microliters or more of blood or other biological fluid. These fluid samples are obtained from a patient, for example, using a needle and syringe, or by lancing a portion of the skin such as the fingertip and "milking" the area to obtain a useful sample volume. These procedures are inconvenient for the patient, and often painful, particularly when frequent samples are required. Less painful methods for obtaining a sample are known such as lancing the arm or thigh, which have a lower nerve ending density. However, lancing the body in the preferred regions typically produces submicroliter samples of blood, because these regions are not heavily supplied with near-surface capillary vessels.

It would therefore be desirable and very useful to develop a relatively painless, easy to use blood analyte sensor, capable of performing an accurate and sensitive analysis of the concentration of analytes in a small volume of sample. Sensors capable of electrochemically measuring an analyte in a sample are known in the art. Some sensors known in the art use at least two electrodes and may contain a redox mediator to aid in the electrochemical reaction. However, the use of an electrochemical sensor for measuring analyte in a small volume introduces error into the measurements. One type of inaccuracy arises from the use of a diffusible redox

chamber, together with any sorbent material, is sized to provide for analysis of a sample volume that is typically no more than about 1 μL, preferably no more than about 0.5 μL, more preferably no more than about 0.25 μL, and most preferably no more than about 0.1 μL. In some instances, the sensor also contains a non-leachable or diffusible second electron transfer agent.

One embodiment of the invention includes a method for determining the concentration of an analyte in a sample by, first, contacting the sample with an electrochemical sensor and then determining the concentration of the analyte. The electrochemical sensor includes a facing electrode pair with a working electrode and a counter electrode and a sample chamber, including a measurement zone, positioned between the two electrodes. The measurement zone is sized to contain no more than about 1 μL of sample.

The invention also includes an electrochemical sensor with two or more facing electrode pairs. Each electrode pair has a working electrode, a counter electrode, and a measurement zone between the two electrodes, the measurement zone being sized to hold no more than about 1 μL of sample. In addition, the sensor also includes a non-leachable redox mediator on the working electrode of at least one of the electrode pairs or a diffusible redox mediator on a surface in the sample chamber or in the sample.

One aspect of the invention is a method of determining the concentration of an analyte in a sample by contacting the sample with an electrochemical sensor and determining the concentration of the analyte by coulometry. The electrochemical sensor includes an electrode pair with a working electrode and a counter electrode. The sensor also includes a sample chamber for holding a sample in electrolytic contact with the working electrode. Within the sample chamber is sorbent material to reduce the volume sample needed to fill the sample chamber so that the sample chamber is sized to contain no more than about 1 μL of sample. The sample chamber also contains a non-leachable or diffusible redox mediator and optionally contains a non-leachable or diffusible second electron transfer agent.

made to the drawings and to the accompanying description, in which there is illustrated and described preferred embodiments of the invention.

Brief Description of the Drawings

Referring now to the drawings, wherein like reference numerals and letters indicate corresponding structure throughout the several views:

Figure 1 is a schematic view of a first embodiment of an electrochemical sensor in accordance with the principles of the present invention having a working electrode and a counter electrode facing each other;

Figure 2 is a schematic view of a second embodiment of an electrochemical sensor in accordance with the principles of the present invention having a working electrode and a counter electrode in a coplanar configuration;

Figure 3 is a schematic view of a third embodiment of an electrochemical sensor in accordance with the principles of the present invention having a working electrode and a counter electrode facing each other and having an extended sample chamber;

Figure 4 is a not-to-scale side-sectional drawing of a portion of the sensor of Figures 1 or 3 showing the relative positions of the redox mediator, the sample chamber, and the electrodes;

Figure 5 is a top view of a fourth embodiment of an electrochemical sensor in accordance with the principles of the present invention, this sensor includes multiple working electrodes;

Figure 6 is a perspective view of an embodiment of an analyte measurement device, in accordance with the principles of the present invention, having a sample acquisition means and the sensor of Figure 4;

Figure 7 is a graph of the charge required to electrooxidize a known quantity of glucose in an electrolyte buffered solution (filled circles) or serum solution (open circles) using the sensor of Figure 1 with glucose oxidase as the second electron transfer agent;

Figure 8 is a graph of the average glucose concentrations for the data of Figure 7 (buffered solutions only) with calibration curves calculated to fit the

Figure 18C illustrates a bottom view of a second film (inverted with respect to Figures 18A and 18B) with counter electrodes placement over the spacer of Figure 18B and first film of Figure 18A;

Figure 19A illustrates a top view of a first film with a working electrode for use in a sixth embodiment of a sensor according to the invention;

Figure 19B illustrates a top view of a spacer for placement on the first film of Figure 19A;

Figure 19C illustrates a bottom view of a second film (inverted with respect to Figures 19A and 19B) with counter electrodes placement over the spacer of Figure 19B and first film of Figure 19A;

Figure 20A illustrates a top view of a first film with a working electrode for use in a seventh embodiment of a sensor according to the invention;

Figure 20B illustrates a top view of a spacer for placement on the first film of Figure 20A;

Figure 20C illustrates a bottom view of a second film (inverted with respect to Figures 20A and 20B) with counter electrodes placement over the spacer of Figure 20B and first film of Figure 20A;

Figure 21A illustrates a top view of a first film with a working electrode for use in a eighth embodiment of a sensor according to the invention;

Figure 21B illustrates a top view of a spacer for placement on the first film of Figure 21A;

Figure 21C illustrates a bottom view of a second film (inverted with respect to Figures 21A and 21B) with counter electrodes placement over the spacer of Figure 21B and first film of Figure 21A;

Figure 22A illustrates a top view of a first film with a working electrode for use in a ninth embodiment of a sensor according to the invention;

Figure 22B illustrates a top view of a spacer for placement on the first film of Figure 22A;

Figure 22C illustrates a bottom view of a second film (inverted with respect to Figures 22A and 22B) with counter electrodes placement over the spacer of Figure 22B and first film of Figure 22A;

Figure 31B illustrates a top view of another embodiment of a sheet of sensor components, according to the invention; and

Figure 32 illustrates a cross-sectional view looking from inside the meter to a sensor of the invention disposed in a meter.

Detailed Description of the Preferred Embodiment

When used herein, the following definitions define the stated term:

An "air-oxidizable mediator" is a redox mediator that is oxidized by air, preferably so that at least 90% of the mediator is in an oxidized state upon storage in air either as a solid or as a liquid during a period of time, for example, one month or less, and, preferably, one week or less, and, more preferably, one day or less.

"Amperometry" includes steady-state amperometry, chronoamperometry, and Cottrell-type measurements.

A "biological fluid" is any body fluid in which the analyte can be measured, for example, blood, interstitial fluid, dermal fluid, sweat, and tears.

The term "blood" in the context of the invention includes whole blood and its cell-free components, such as, plasma and serum.

"Coulometry" is the determination of charge passed or projected to pass during complete or nearly complete electrolysis of the analyte, either directly on the electrode or through one or more electron transfer agents. The charge is determined by measurement of charge passed during partial or nearly complete electrolysis of the analyte or, more often, by multiple measurements during the electrolysis of a decaying current and elapsed time. The decaying current results from the decline in the concentration of the electrolyzed species caused by the electrolysis.

A "counter electrode" refers to one or more electrodes paired with the working electrode, through which passes an electrochemical current equal in magnitude and opposite in sign to the current passed through the working electrode. The term "counter electrode" is meant to include counter electrodes which also function as reference electrodes (i.e. a counter/reference electrode) unless the

A "non-diffusible," "non-leachable," or "non-releasable" compound is a compound which does not substantially diffuse away from the working surface of the working electrode for the duration of the analyte assay.

The "potential of the counter/reference electrode" is the half cell potential of the reference electrode or counter/reference electrode of the cell when the solution in the cell is 0.1 M NaCl solution at pH7.

"Potentiometry" and "chronopotentiometry" refer to taking a potentiometric measurement at one or more points in time.

A "redox mediator" is an electron transfer agent for carrying electrons between the analyte and the working electrode, either directly, or via a second electron transfer agent.

A "reference electrode" includes a reference electrode that also functions as a counter electrode (i.e., a counter/reference electrode) unless the description provides that a "reference electrode" excludes a counter/reference electrode.

A "second electron transfer agent" is a molecule that carries electrons between a redox mediator and the analyte.

"Sorbent material" is material that wicks, retains, and/or is wetted by a fluid sample and which typically does not substantially prevent diffusion of the analyte to the electrode.

A "surface in the sample chamber" includes a surface of a working electrode, counter electrode, counter/reference electrode, reference electrode, indicator electrode, a spacer, or any other surface bounding the sample chamber.

A "working electrode" is an electrode at which analyte is electrooxidized or electroreduced with or without the agency of a redox mediator.

A "working surface" is the portion of a working electrode that is covered with non-leachable redox mediator and exposed to the sample, or, if the redox mediator is diffusible, a "working surface" is the portion of the working electrode that is exposed to the sample.

The small volume, *in vitro* analyte sensors of the present invention are designed to measure the concentration of an analyte in a portion of a sample

used, a dielectric 40 may be deposited on the electrode over, under, or surrounding the region with the redox mediator, as shown in Figure 4. Suitable dielectric materials include waxes and non-conducting organic polymers such as polyethylene. Dielectric 40 may also cover a portion of the redox mediator on the electrode. The covered portion of the redox mediator will not contact the sample, and, therefore, will not be a part of the electrode's working surface.

Sensing Chemistry

In addition to the working electrode 22, sensing chemistry materials are provided in the sample chamber 26 for the analysis of the analyte. This sensing chemistry preferably includes a redox mediator and a second electron transfer mediator, although in some instances, one or the other may be used alone. The redox mediator and second electron transfer agent can be independently diffusible or non-leachable (i.e., non-diffusible) such that either or both may be diffusible or non-leachable. Placement of sensor chemistry components may depend on whether they are diffusible or non-leachable. For example, non-leachable and/or diffusible component(s) typically form a sensing layer on the working electrode. Alternatively, one or more diffusible components may be disposed on any surface in the sample chamber prior to the introduction of the sample. As another example, one or more diffusible component(s) may be placed in the sample prior to introduction of the sample into the sensor.

If the redox mediator is non-leachable, then the non-leachable redox mediator is typically disposed on the working electrode 22 as a sensing layer 32. In an embodiment having a redox mediator and a second electron transfer agent, if the redox mediator and second electron transfer agent are both non-leachable, then both of the non-leachable components are disposed on the working electrode 22 as a sensing layer 32.

If, for example, the second electron transfer agent is diffusible and the redox mediator is non-leachable, then at least the redox mediator is disposed on the working electrode 22 as a sensing layer 32. The diffusible second electron transfer agent need not be disposed on a sensing layer of the working electrode, but may be

Similarly, the diffusible redox mediator and/or second electron transfer agent may first dissolve from the surface on which it was placed as a solid and then the diffusible redox mediator and/or second electron transfer agent may diffuse into the sample, either rapidly or over a period of time. If the redox mediator and/or second electron transfer agent diffuse over a period of time, a user may be directed to wait a period of time before measuring the analyte concentration to allow for diffusion of the redox mediator and/or second electron transfer agent.

Background Signal

In at least some instances, a diffusible redox mediator may shuttle back and forth from the working electrode to the counter electrode even in the absence of analyte. This typically creates a background signal. For coulometric measurements, this background signal is referred to herein as "Q_{Back}." The background signal corresponds to the charge passed in an electrochemical assay in the absence of the analyte. The background signal typically has both a transient component and a steady-state component. At least a portion of the transient component may result, for example, from the establishment of a concentration gradient of the mediator in a particular oxidation state. At least a portion of the steady-state component may result, for example, from the redox mediator shuttling between the working electrode and counter or counter/reference electrode. Shuttling refers to the redox mediator being electrooxidized (or electroreduced) at the working electrode and then being electroreduced (or electrooxidized) at the counter or counter/reference electrode, thereby making the redox mediator available to be electrooxidized (or electroreduced) again at the working electrode so that the redox mediator is cycling between electrooxidation and electroreduction.

The amount of shuttling of the redox mediator, and therefore, the steady-state component of the background signal varies with, for example, the effective diffusion coefficient of the redox mediator, the viscosity of the sample, the temperature of the sample, the dimensions of the electrochemical cell, the distance between the working electrode and the counter or counter/reference electrode, and

electrode by the shuttling of the redox mediator and the charge transferred at the working electrode by the electrolysis of the analyte.

The size of the background signal may be compared to a predetermined amount of analyte. The predetermined amount of analyte in a sample may be, for example, an expected or average molar amount of analyte. The expected or average molar amount of analyte may be determined as, for example, the average value for users or individuals; an average value for a population; a maximum, minimum, or average of a normal physiological range; a maximum or minimum physiological value for a population; a maximum or minimum physiological value for users or individuals; an average, maximum, or minimum deviation outside a normal physiological range value for users, individuals, or a population; a deviation above or below an average value for a population; or an average, maximum, or minimum deviation above or below an average normal physiological value for users or individuals. A population may be defined by, for example, health, sex, or age, such as, for example, a normal adult, child, or newborn population. If a population is defined by health, the population may include people lacking a particular condition or alternatively, having a particular condition, such as, for example, diabetes. Reference intervals pertaining to average or expected values, such as, for example, those provided in Tietz Textbook of Clinical Chemistry, Appendix (pp. 2175-2217) (2nd Ed., Carl A. Burtis and Edward R. Ashwood, eds., W.D. Saunders Co., Philadelphia 1994) (incorporated herein by reference) may be used as guidelines, but a physical examination or blood chemistry determination by a skilled physician may also be used to determine an average or expected value for an individual. For example, an adult may have glucose in a concentration of 65 to 95 mg/dL in whole blood or L-lactate in a concentration of 8.1 to 15.3 mg/dL in venous whole blood after fasting, according to Tietz Textbook of Clinical Chemistry. An average normal physiological concentration for an adult, for example, may then correspond to 80 mg/dL for glucose or 12.7 mg/dL for lactate. Other examples include a person having juvenile onset diabetes, yet good glycemic control, and a glucose concentration between about 50 mg/dL and 400 mg/dL, thereby having an average molar amount of 225 mg/dL. In another instance, a non-diabetic adult may

no less than about -100 mV relative to the potential of a reference or counter/reference electrode. A suitable reference or counter/reference electrode (e.g., a silver/silver chloride electrode) may be chosen. Preferably, the redox mediator a) oxidizes the analyte at a half wave potential, as measured by cyclic voltammetry in 0.1 M NaCl at pH 7, of no more than about +50 mV, +25 mV, 0 mV, -25 mV, -50 mV, -100 mV, or -150 mV relative to the potential of the reference or counter/reference electrode or b) reduces the analyte at a half wave potential, as measured by cyclic voltammetry in 0.1 M NaCl at pH 7, of no less than about -50 mV, -25 mV, 0 mV, +25 mV, +50 mV, +100 mV, +150 mV, or +200 mV relative to the potential of the reference or counter/reference electrode. Alternatively, in the case of reduction of the redox mediator by the counter electrode, the sensor is operated at an applied potential of no more than about +100 mV, +50 mV, +25 mV, 0 mV, -25 mV, -50 mV, -100 mV, or –150 mV between the working electrode and the counter or counter/reference electrode. In the case of oxidation of the redox mediator at the counter electrode, the sensor is operated at an applied potential of no less than about -100 mV, -50 mV, -25 mV, 0 mV, +25 mV, +50 mV, +100 mV, +150 mV, or +200 mV between the working electrode and the counter or counter/reference electrode.

Another method includes controlling the applied potential such that for an electrooxidative assay the redox mediator is not readily reduced at the counter or counter/reference electrode or for an electroreductive assay the redox mediator is not readily oxidized at the counter or counter/reference electrode. This can be accomplished, for example, in an electrooxidative assay by using a sensor having a diffusible redox mediator with a potential, relative to a reference or counter/reference electrode, that is negative with respect to the potential of the counter electrode (relative to a reference electrode) or the counter/reference electrode. The potential (relative to a reference or counter/reference electrode) of the working electrode is chosen to be positive with respect to the redox mediator and may be negative with respect to the counter or counter/reference electrode, so that the redox mediator is oxidized at the working electrode. For example, when the electrooxidation of an analyte is mediated by a diffusible redox mediator with a

minimum deviation outside a normal physiological range value for users, individuals, or a population; a deviation above or below an average value for a population; or an average, maximum, or minimum deviation above or below an average normal physiological value for users or individuals. A population may be defined by, for example, health, sex, or age, such as, for example, a normal adult, child, or newborn population. If a population is defined by health, the population may include people lacking a particular condition or alternatively, having a particular condition, such as, for example, diabetes. Reference intervals pertaining to average or expected values, such as, for example, those provided in Tietz Textbook of Clinical Chemistry, *supra*, may be used as guidelines, but a physical examination or blood chemistry determination may also determine an average or expected value. For example, the physiological average molar amount of analyte may depend on the health or age of the person from whom the sample is obtained. This determination is within the knowledge of a skilled physician.

By reducing the concentration of the redox mediator relative to the concentration of the analyte, the signal attributable to the analyte relative to the signal attributable to the shuttling of the redox mediator is increased. In implementation of this method, the molar amount of redox mediator may be no more than 50%, 20%, 10%, or 5%, on a stoichiometric basis, of the expected or average molar amount of analyte.

The amount of redox mediator used in such a sensor configuration should fall within a range. The upper limit of the range may be determined based on, for example, the acceptable maximum signal due to shuttling of the redox mediator; the design of the electrochemical cell, including, for example, the dimensions of the cell and the position of the electrodes; the effective diffusion coefficient of the redox mediator; and the length of time needed for the assay. Moreover, the acceptable maximum signal due to redox mediator shuttling may vary from assay to assay as a result of one or more assay parameters, such as, for example, whether the assay is intended to be qualitative, semi-quantitative, or quantitative; whether small differences in analyte concentration serve as a basis to modify therapy; and the expected concentration of the analyte.

functional groups to prevent or reduce the flow of a charged redox mediator relative to the flow of a charge neutral or less charged analyte. If the redox mediator is positively charged, as are many of the osmium redox mediators described below, the barrier can be a positively charged or polar film, such as a methylated poly(1-vinyl imidazole). If the redox mediator is negatively charged, the barrier can be a negatively charged or polar film, such as Nafion®. Examples of suitable polar matrices include a bipolar membrane, a membrane having a cationic polymer cross-linked with an anionic polymer, and the like. In some instances, the barrier reduces the oxidation or reduction of the diffusible redox mediator at the counter electrode by at least 25%, 50%, or 90%.

Still another sensor configuration for limiting the background current includes a sensor having a redox mediator that is more readily oxidized or reduced on the working electrode than reduced or oxidized on the counter electrode. The rate of reaction of the redox mediator at an electrode can be a function of the material of the electrode. For example, some redox mediators may react faster at a carbon electrode than at a Ag/AgCl electrode. Appropriate selection of the electrodes may provide a reaction rate at one electrode that is significantly slower than the rate at the other electrode. In some instances, the rate of oxidation or reduction of the diffusible redox mediator at the counter electrode is reduced by at least 25%, 50%, or 90%, as compared to the working electrode. In some instances the rate of reaction for the redox mediator at the counter or counter/reference electrode is controlled by, for example, choosing a material for the counter or counter/reference electrode that would require an overpotential or a potential higher than the applied potential to increase the reaction rate at the counter or counter/reference electrode.

Another sensor configuration for limiting background current includes elements suitable for reducing the diffusion of the redox mediator. Diffusion can be reduced by, for example, using a redox mediator with a relatively low diffusion coefficient or increasing the viscosity of the sample in the measurement zone. In another embodiment, the diffusion of the redox mediator may be decreased by choosing a redox mediator with high molecular weight, such as, for

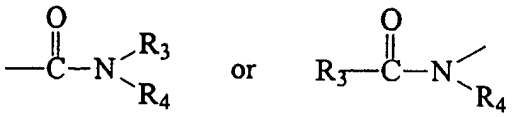
and derivatives thereof, wherein R_1 and R_2 are each independently hydrogen, hydroxy, alkyl, alkoxy, alkenyl, vinyl, allyl, amido, amino, vinylketone, keto, or sulfur-containing groups.

The term "alkyl" includes a straight or branched saturated aliphatic hydrocarbon chain having from 1 to 6 carbon atoms, such as, for example, methyl, ethyl isopropyl (1-methylethyl), butyl, *tert*-butyl (1,1-dimethylethyl), and the like. Preferably the hydrocarbon chain has from 1 to 3 carbon atoms.

The term "alkoxy" includes an alkyl as defined above joined to the remainder of the structure by an oxygen atom, such as, for example, methoxy, ethoxy, propoxy, isopropoxy (1-methylethoxy), butoxy, *tert*-butoxy, and the like.

The term "alkenyl" includes an unsaturated aliphatic hydrocarbon chain having from 2 to 6 carbon atoms, such as, for example, ethenyl, 1-propenyl, 2-propenyl, 1-butenyl, 2-methy-1-propenyl, and the like. Preferably the hydrocarbon chain has from 2 to 3 carbon atoms.

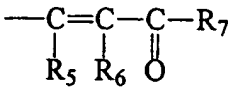
The term "amido" includes groups having a nitrogen atom bonded to the carbon atom of a carbonyl group and includes groups having the following formulas:



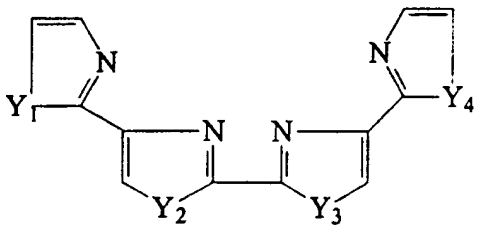
wherein R_3 and R_4 are each independently hydrogen, alkyl, alkoxy, or alkenyl.

The term "amino" as used herein includes alkylamino, such as methylamino, diethylamino, N,N-methylethylamino and the like; alkoxyalkylamino, such as N-(ethoxyethyl)amino, N,N-di(methoxyethyl)amino, N,N-(methoxyethyl)(ethoxyethyl)amino, and the like; and nitrogen-containing rings, such as piperidino, piperazino, morpholino, and the like.

The term "vinylketone" includes a group having the formula:



wherein R_5, R_6, and R_7 are each independently hydrogen, alkyl, alkoxy, or alkenyl.



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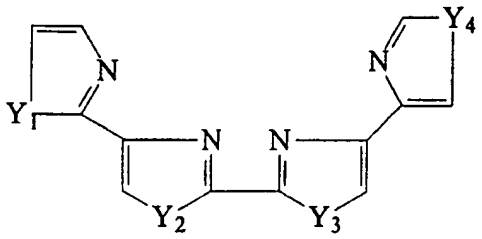

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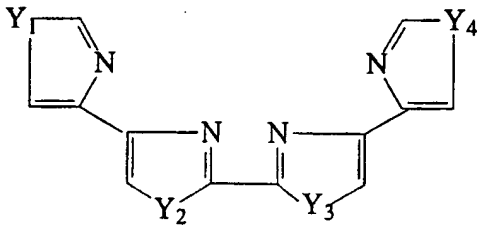
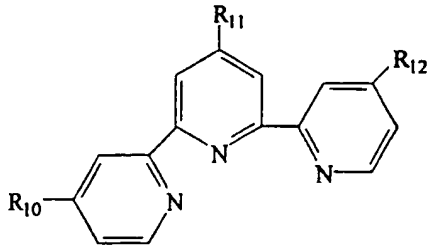


(XII)

wherein Y_1, Y_2, Y_3, and Y_4 are each independently an oxygen atom, a sulfur atom, a selenium atom, or a substituted nitrogen atom having the formula NR_9 wherein R_9 is hydrogen, hydroxy, alkyl, alkoxy, alkenyl, amido, amino, vinylketone, keto, or sulfur-containing group. The terms "alkyl," "alkoxy," "alkenyl," "amido," "amino," "vinylketone," "keto," and "sulfur-containing group" are as defined above.

Suitable derivatives of these ligands include, for example, the addition of alkyl, alkoxy, alkenyl, vinylester, and amido functional groups to any of the available sites on the heterocyclic ring, including, for example, on the 4-position (i.e., para to nitrogen) of the pyridine rings or on one of the nitrogen atoms of the imidazole ring.

Suitable derivatives of 2,2'-bipyridine for complexation with the osmium cation include, for example, mono-, di-, and polyalkyl-2,2'-bipyridines, such as 4,4'-dimethyl-2,2'-bipyridine; mono-, di-, and polyalkoxy-2,2'-bipyridines, such as 4,4'-dimethoxy-2,2'-bipyridine and 2,6'-dimethoxy-2,2'-bipyridine; mono-, di-, and polyacetamido-2,2'-bipyridines, such as 4,4'-di(acetamido)-2,2'-bipyridine; mono-, di-, and polyalkylaminoalkoxy-2,2'-bipyridines, such as 4,4'-di(N,N-dimethylaminoethoxy)-2,2'-bipyridine; and substituted mono-, di-, and

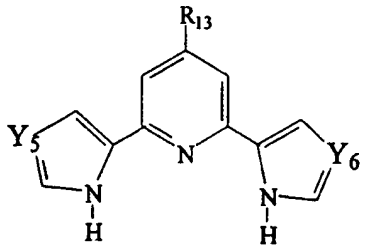


(XIII)

wherein R_{10}, R_{11}, and R_{12} are each independently hydrogen, hydroxy, alkyl, alkoxy, alkenyl, vinyl, allyl, amido, amino, vinylketone, keto, or sulfur-containing group.

The terms "alkyl," "alkoxy," "alkenyl," "amido," "amino," "vinylketone," "keto," and "sulfur-containing group" are as defined above.

Other suitable redox mediator derivatives include compounds of the formula:

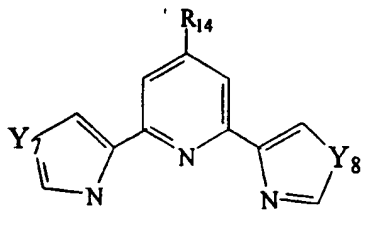


(XIV)

wherein R_{13} is hydrogen, hydroxy, alkyl, alkoxy, alkenyl, vinyl, allyl, vinylketone, keto, amido, amino, or sulfur-containing group; and Y_5 and Y_6 are each independently a nitrogen or carbon atom.

The terms "alkyl," "alkoxy," "alkenyl," "amido," "amino," "vinylketone," "keto," and "sulfur-containing group" are as defined above.

Still other suitable derivatives include compounds of the formula:



(XV)

wherein R_{14} is as defined above and Y_7 and Y_8 are each independently a sulfur or oxygen atom.

Examples of suitable redox mediators also include, for example, osmium cations complexed with (a) two bidentate ligands, such as 2,2'-bipyridine,

the potential; and Os(terpyridine)$(L)_2Cl$, where L is an aminopyridine, such as a dialkylaminopyridine; an N-substituted imidazole, such as N-methyl imidazole; an oxazole; a thiazole; or an alkoxypyridine, such as methoxypyridine. X is halogen as described above.

Osmium-free diffusible redox mediators include, for example, phenoxazines, such as, 7-dimethylamino-1,2-benzophenoxazine (Meldola Blue), 1,2-benzophenoxazine, and Nile Blue; 3-β-naphthoyl (Brilliant Cresyl Blue); tetramethylphenylenediamine (TMPD); dichlorophenolindophenol (DCIP); N-methyl phenazonium salts, for example, phenazine methosulfate (PMS), N-methylphenazine methosulfate and methoxyphenazine methosulfate; tetrazolium salts, for example, tetrazolium blue or nitrotetrazolium blue; and phenothiazines, for example, toluidine blue O.

Examples of other redox species include stable quinones and species that in their oxidized state have quinoid structures, such as Nile Blue and indophenol. Examples of suitable quinones include, for example, derivatives of naphthoquinone, phenoquinone, benzoquinone, naphthenequinone, and the like. Examples of naphthoquinone derivatives include juglone (i.e., 5-hydroxy-1,4-naphthoquinone) and derivatives thereof, such as, for example, 2,3-dichloro-5,8-dihydroxy-1,4-naphthoquinone, 2,3-dimethyl-5,8-dihydroxy-1,4-naphthoquinone, 2-chloro-5,8-dihydroxy-1,4-naphthoquinone, 2,3-methoxy-5-hydroxy-1,4-naphthoquinone, and the like. Other examples include aminonaphthoquinones, such as, for example, morpholino-naphthoquinones, such as 2-chloro-3-morpholino-1,4-naphthoquinone; piperidino-naphthoquinones, such as 2-methyl-3-peperidino-1,4-naphthoquinone; piperazino-naphthoquinones, such as 2-ethoxy-3-piperazino-1,4-naphthoquinone; and the like.

Suitable phenoquinone derivatives include, for example, coerulignone (i.e., 3,3',5,5'-tetramethoxydiphenoquinone) and derivatives thereof, such as, for example, 3,3',5,5'-tetramethyldiphenoquinone, 3,3',5,5'-tetrahydroxydiphenoquinone, and the like.

Suitable benzoquinone derivatives include, for example, coenzyme Q_0 (i.e., 2,3-dimethoxy-5-methyl-1,4-benzoquinone) and derivatives thereof, such as,

to form a redox polymer have nitrogen-containing heterocycles, such as pyridine, imidazole, or derivatives thereof for binding as ligands to the redox species. Suitable polymers for complexation with redox species, such as the transition metal complexes, described above, include, for example, polymers and copolymers of poly(1-vinyl imidazole) (referred to as "PVI") and poly(4-vinyl pyridine) (referred to as "PVP"), as well as polymers and copolymers of poly(acrylic acid) or polyacrylamide that have been modified by the addition of pendant nitrogen-containing heterocycles, such as pyridine and imidazole. Modification of poly(acrylic acid) may be performed by reaction of at least a portion of the carboxylic acid functionalities with an aminoalkylpyridine or aminoalkylimidazole, such as 4-ethylaminopyridine, to form amides. Suitable copolymer substituents of PVI, PVP, and poly(acrylic acid) include acrylonitrile, acrylamide, acrylhydrazide, and substituted or quaternized 1-vinyl imidazole. The copolymers can be random or block copolymers.

The transition metal complexes of non-leachable redox polymers are typically covalently or coordinatively bound with the nitrogen-containing heterocycles (e.g., imidazole and/or pyridine rings) of the polymer. The transition metal complexes may have vinyl functional groups through which the complexes can be co-polymerized. Suitable vinyl functional groups include, for example, vinylic heterocycles, amides, nitriles, carboxylic acids, sulfonic acids, or other polar vinylic compounds. An example of a redox polymer of this type is poly(vinyl ferrocene) or a derivative of poly(vinyl ferrocene) functionalized to increase swelling of the redox polymer in water.

Another type of redox polymer contains an ionically-bound redox species, by forming multiple ion-bridges. Typically, this type of mediator includes a charged polymer coupled to an oppositely charged redox species. Examples of this type of redox polymer include a negatively charged polymer such as Nafion® (DuPont) coupled to multiple positively charged redox species such as an osmium or ruthenium polypyridyl cation. Another example of an ionically-bound mediator is a positively charged polymer such as quaternized poly(4-vinyl pyridine) or poly(1-vinyl imidazole) coupled to a negatively charged redox species such as ferricyanide

amines or other nitrogen compounds. These cross-linking agents include carbodiimides or compounds with active N-hydroxysuccinimide or imidate functional groups. Yet other examples of cross-linking agents are quinones (e.g., tetrachlorobenzoquinone and tetracyanoquinodimethane) and cyanuric chloride. Other cross-linking agents may also be used. In some embodiments, an additional cross-linking agent is not required. Further discussion and examples of cross-linking and cross-linking agents are found in U.S. Patents Nos. 5,262,035; 5,262,305; 5,320,725; 5,264,104; 5,264,105; 5,356,786; and 5,593,852, herein incorporated by reference.

In another embodiment, the redox polymer is immobilized by the functionalization of the electrode surface and then the chemical bonding, often covalently, of the redox polymer to the functional groups on the electrode surface. One example of this type of immobilization begins with a poly(4-vinyl pyridine). The polymer's pyridine rings are, in part, complexed with a reducible/oxidizable species, such as $[Os(bpy)_2Cl]^{+/2+}$ where bpy is 2,2'-bipyridine. Part of the pyridine rings are quaternized by reaction with 2-bromoethylamine. The polymer is then crosslinked, for example, using a diepoxide, such as poly(ethylene glycol) diglycidyl ether.

Carbon surfaces can be modified for attachment of a redox polymer, for example, by electroreduction of a diazonium salt. As an illustration, reduction of a diazonium salt formed upon diazotization of p-aminobenzoic acid modifies a carbon surface with phenylcarboxylic acid functional groups. These functional groups can be activated by a carbodiimide, such as 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC). The activated functional groups are bound with an amine-functionalized redox couple, such as, for example, the quaternized osmium-containing redox polymer described above or 2-aminoethylferrocene, to form the redox couple.

Similarly, gold and other metal surfaces can be functionalized by, for example, an amine, such as cystamine, or by a carboxylic acid, such as thioctic acid. A redox couple, such as, for example, $[Os(bpy)_2(pyridine\text{-}4\text{-}carboxylate)Cl]^{0/+}$, is activated by 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC)

Second Electron Transfer Agent

In a preferred embodiment of the invention, the sensor includes a redox mediator and a second electron transfer agent which is capable of transferring electrons to or from the redox mediator and the analyte. The second electron transfer agent may be diffusible or may be non-leachable (e.g., entrapped in or coordinatively, covalently, or ionically bound to the redox polymer). One example of a suitable second electron transfer agent is an enzyme which catalyzes a reaction of the analyte. For example, a glucose oxidase or glucose dehydrogenase, such as pyrroloquinoline quinone glucose dehydrogenase (PQQ), is used when the analyte is glucose. A lactate oxidase fills this role when the analyte is lactate. Other enzymes can be used for other analytes. These enzymes catalyze the electrolysis of an analyte by transferring electrons between the analyte and the electrode via the redox mediator. In some embodiments, the second electron transfer agent is non-leachable, and more preferably immobilized on the working electrode, to prevent unwanted leaching of the agent into the sample. This is accomplished, for example, by cross-linking the non-leachable second electron transfer agent with the non-leachable redox mediator, thereby providing a sensing layer with non-leachable components on the working electrode. In other embodiments, the second electron transfer agent is diffusible (and may be disposed on any surface of the sample chamber or placed in the sample).

Counter Electrode

Counter electrode 24, as illustrated in Figures 1-4, may be constructed in a manner similar to working electrode 22. Counter electrode 24 may also be a counter/reference electrode. Alternatively, a separate reference electrode may be provided in contact with the sample chamber. Suitable materials for the counter/reference or reference electrode include Ag/AgCl or Ag/AgBr printed on a non-conducting base material or silver chloride on a silver metal base. The same materials and methods may be used to make the counter electrode as are available for constructing the working electrode 22, although different materials and methods

the region 21 of the overlapping electrodes 22, 24 and the dielectric constant of the material between the electrodes (e.g., air or a sorbent material) are known, then the separation between the electrodes can be calculated to determine the volume of the measurement zone.

Figure 11A illustrates one embodiment of the invention in which the electrodes 22, 24 are positioned in a facing arrangement. For the capacitance to be uniform among similarly constructed analyte sensors having this particular sensor configuration, the registration (i.e., the positioning of the two electrodes relative to one another) should be uniform. If the position of either of the electrodes is shifted in the x-y plane from the position shown in Figure 11A, the size of the overlap, and therefore, of the capacitance, will change. The same principle holds for the volume of the measurement zone.

Figures 11B and 11C illustrate other embodiments of the invention with electrodes 22, 24 in a facing arrangement. In these particular arrangements, the position of either of the electrodes may be shifted, by at least some minimum distance, in the x-y plane relative to the other electrode without a change in the capacitance or the volume of the measurement zone. In these electrode arrangements, each electrode 22, 24 includes an arm 122, 124, respectively, which overlaps with the corresponding arm of the other electrode. The two arms 122, 124 are not parallel to each other (such as illustrated in Figure 11A); rather, the arms 122, 124 are disposed at an angle 123, which is greater than zero, relative to each other. In addition, the two arms 122, 124 extend beyond the region 21 of overlap (i.e., each arm has extra length corresponding to the difference between the length of the arm 222, 224, respectively, and the width 121 of the overlap 21). With these electrode arrangements, there can be a certain amount of allowed imprecision in the registration of the electrodes 22, 24 which does not change the capacitance of the electrode arrangement. A desired amount of allowed imprecision in the registration can be designed into the electrode arrangement by varying the angle 123 at which the arms 122, 124 overlap and the size of the extra length of each arm 122, 124 relative to the width 121 of the region 21 of overlap. Typically, the closer that the arms 122, 124 are to being perpendicular (i.e., angle 123 is 90°), the greater the

A spacer 28 can be used to keep the electrodes apart when the electrodes face each other as depicted in Figures 1 and 3. The spacer is typically constructed from an inert non-conducting material such as pressure-sensitive adhesive, polyester, Mylar™, Kevlar™ or any other strong, thin polymer film, or, alternatively, a thin polymer film such as a Teflon™ film, chosen for its chemical inertness. In addition to preventing contact between the electrodes, the spacer 28 often functions as a portion of the boundary for the sample chamber 26 as shown in Figures 1- 4. Other spacers include layers of adhesive and double-sided adhesive tape (e.g., a carrier film with adhesive on opposing sides of the film).

Sample Chamber

The sample chamber 26 is typically defined by a combination of the electrodes 22, 24, an inert base 30, and a spacer 28 as shown in Figures 1-4. A measurement zone is contained within this sample chamber and is the region of the sample chamber that contains only that portion of the sample that is interrogated during the analyte assay. In the embodiment of the invention illustrated in Figures 1 and 2, sample chamber 26 is the space between the two electrodes 22, 24 and/or the inert base 30. In this embodiment, the sample chamber has a volume that is preferably no more than about 1 μL, more preferably no more than about 0.5 μL, and most preferably no more than about 0.25 μL. In the embodiment of the invention depicted in Figures 1 and 2, the measurement zone has a volume that is approximately equal to the volume of the sample chamber. In a preferred embodiment the measurement zone includes 80% of the sample chamber, 90% in a more preferred embodiment, and about 100% in a most preferred embodiment.

In another embodiment of the invention, shown in Figure 3, sample chamber 26 includes much more space than the region proximate electrodes 22, 24. This configuration makes it possible to provide multiple electrodes in contact with one or more sample chambers, as shown in Figure 5. In this embodiment, sample chamber 26 is preferably sized to contain a volume of no more than about 1 μL, more preferably no more than about 0.5 μL, and most preferably no more than about 0.25 μL. The measurement zone (i.e., the region containing the volume of sample to

non-conducting base material 102 so that at least a portion of the conducting layer 100 is included in the recess 104. The recess 104 may be formed using a variety of techniques including indenting, deforming, or otherwise pushing in the base material 102. One additional exemplary method for forming the recess includes embossing the base material 102. For example, the base material 102 may be brought into contact with an embossing roll or stamp having raised portions, such as punch members or channels, to form the recess 104. In some embodiments, the base material 102 may be heated to soften the material.

The recess 104 may be circular, oval, rectangular, or any other regular or irregular shape. Alternatively, the recess 104 may be formed as a channel which extends along a portion of the base material 102. The conducting layer 100 may extend along the entire channel or only a portion of the channel. The measurement zone may be restricted to a particular region within the channel by, for example, depositing the sensing layer 32 on only that portion of the conducting layer 100 within the particular region of the channel. Alternatively, the measurement zone may be defined by placing a second electrode 107 over only the desired region of the first electrode 105.

At least a portion, and in some cases, all, of the conducting layer 100 is situated in the recess 104. This portion of the conducting layer 100 may act as a first electrode 105 (a counter electrode or a working electrode). If the conducting layer 100 forms the working electrode, then a sensing layer 32 may be formed over a portion of the conducting layer 100 by depositing a non-leachable redox mediator and/or second electron transfer agent in the recess 104, as shown in Figure 12B. If a diffusible redox mediator or second electron transfer agent is used, then the diffusible material may be disposed on any surface in the sample chamber or in the sample.

A second electrode 107 is then formed by depositing a second conducting layer on a second base material 106. This second electrode 107 is then positioned over the first electrode 105 in a facing arrangement. Although not illustrated, if the redox mediator is non-leachable it will be understood that if the first electrode 105 were to function as a counter electrode, then the sensing layer 32

The conductive ink typically contains metal or carbon dissolved or dispersed in a solvent or dispersant. When the solvent or dispersant is removed, the metal or carbon forms a conductive layer 110 that can then be used as a first electrode 115. A second electrode 117 can be formed on a second base material 116 and positioned over the recess 114, as described above. In embodiments having a non-leachable redox mediator, a sensing layer 32 is formed on the first electrode 115 to form a working electrode, as shown in Figure 13B. In other embodiments having a non-leachable redox mediator, the sensing layer 32 may be formed on the second electrode 117 to form a working electrode. Alternatively, if a diffusible redox mediator is used, then the working electrode need not include the sensing layer disposed thereon. In fact, no sensing layer is required because the redox mediator may be placed in the sample and likewise for a diffusible second electron transfer agent, if one is present. Any diffusible components may be independently disposed on any surface of the sample chamber or placed in the sample. Furthermore, a sorbent material (not shown) may be formed within the recess, for example, on the first electrode 115.

A binder, such as a polyurethane resin, cellulose derivative, elastomer (e.g., silicones, polymeric dienes, or acrylonitrile-butadiene-styrene (ABS) resins), highly fluorinated polymer, or the like, may also be included in the conductive ink. Curing the binder may increase the conductivity of the conductive layer 110, however, curing is not necessary. The method of curing the binder may depend on the nature of the particular binder that is used. Some binders are cured by heat and/or ultraviolet light.

These structures allow for the formation of electrochemical sensors in which the volume of the measurement zone depends, at least in part, on the accuracy and reproducibility of the recess 104. Embossing, laser etching, photolithographic etching and other methods can be used to make reproducible recesses 104, even on the scale of 200 μm or less.

in the sensor, so that a sample may be brought into contact with tab 33, sorbed by the tab, and conveyed into the sample chamber 26 by the wicking action of the sorbent material 34. This provides a preferred method for directing the sample into the sample chamber 26. For example, the sensor may be brought into contact with a region of an animal (including human) that has been pierced with a lancet to draw blood. The blood is brought in contact with tab 33 and drawn into sample chamber 26 by the wicking action of the sorbent 34. The direct transfer of the sample to the sensor is especially important when the sample is very small, such as when the lancet is used to pierce a portion of the animal that is not heavily supplied with near-surface capillary vessels and furnishes a blood sample volume of 1 μL or less.

Methods other than the wicking action of a sorbent may be used to transport the sample into the sample chamber or measurement zone. Examples of such methods for transport include the application of pressure on a sample to push it into the sample chamber, the creation of a vacuum by a pump or other vacuum-producing method in the sample chamber to pull the sample into the chamber, capillary action due to interfacial tension of the sample with the walls of a thin sample chamber, as well as the wicking action of a sorbent material.

The sensor can also be used in conjunction with a flowing sample stream. In this configuration, the sample stream is made to flow through a sample chamber. The flow is stopped periodically and the concentration of the analyte is determined by an electrochemical method, such as coulometry. After the measurement, the flow is resumed, thereby removing the sample from the sensor. Alternatively, sample may flow through the chamber at a very slow rate, such that all of the analyte is electrolyzed in transit, yielding a current dependent only upon analyte concentration and flow rate.

Other filler materials may be used to fill the measurement zone and reduce the sample volume. For example, glass beads can be deposited in the measurement zone to occupy space. Preferably, these filler materials are hydrophilic so that the body fluid can easily flow into the measurement zone. In some cases, such as glass beads with a high surface area, these filler materials may also wick the

times larger. The non-leachable or diffusible redox mediator and/or second electron transfer agent can be provided on either the first or second substrates 500, 508 in a region corresponding to the channel 506, as described above.

The working electrode and counter electrodes can be formed to cover the entire channel region (except for a small space between the two counter electrodes). In this embodiment, the sample chamber and measurement zone are effectively the same and have the same volume. In other embodiments, the measurement zone has, for example, 80% or 90% of the volume of the sample chamber. It will be understood that similar sensors could be made using one counter electrode or three or more counter electrodes. It will also be understood that multiple working electrodes may also be provided on the sensor.

One example of a method for making the thin film sensors is described with respect to the sensor arrangement displayed in Figures 18A to 18C and can be used to make a variety of other sensor arrangements, including those described before. A substrate, such as a plastic substrate, is provided. The substrate can be an individual sheet or a continuous roll on a web. This substrate can be used to make a single sensor or to make multiple sensors. The multiple sensors can be formed on a substrate 1000 as working electrodes 1010 and counter electrode(s) 1020. In some embodiments, the substrate can be scored and folded to bring the working electrodes 1010 and counter electrodes 1020 together to form the sensor. In some embodiments, as illustrated in Figure 31A, the individual working electrodes 1010 (and, in a separate section, the counter electrode(s) 1020) can be formed next to each other on the substrate 1000, to reduce waste material, as illustrated in Figure 31A. In other embodiments, the individual working electrodes 1010 (and, in a separate section, the counter electrode(s) 1020) can be spaced apart, as illustrated in Figure 31B. The remainder of the process is described for the manufacture of multiple sensors, but can be readily modified to form individual sensors.

Carbon or other electrode material (e.g., metal, such as gold or platinum) is formed on the substrate to provide a working electrode for each sensor. The carbon or other electrode material can be deposited by a variety of methods including printing a carbon or metal ink, vapor deposition, and other methods.

may be a single layer of adhesive or a double-sided adhesive tape (e.g., a polymer carrier film with adhesive disposed on opposing surfaces). To form the channel, the spacer, optionally provided with one or more release liners, may be cut (e.g., die-cut) to remove the portion of the adhesive corresponding to the channel prior to disposing the spacer on the substrate. Alternatively, the adhesive may be printed or otherwise disposed on the substrate according to a pattern which defines the channel region. The thickness of the spacer typically determines the spacing between the working and counter electrodes. When the uniformity of this spacing among sensors is necessary (e.g., for coulometric measurements), uniformity in the thickness of the spacer is important. Preferably, the thickness does not vary more than $\pm$ 5% over the individual sensor and/or among individual sensors in a batch.

The non-leachable or diffusible redox mediator and/or second electron transfer agent are disposed onto the substrate in at least the sample chamber region. If either or both of these components is non-leachable, that component or components must be disposed on the working electrode. If either or both of these components is diffusible, that component or components can be disposed on any surface of the substrate in the channel region. The redox mediator and/or second electrode transfer agent can be disposed independently or together on the substrate prior to or after disposition of the spacer. The redox mediator and/or second electrode transfer agent may be disposed by a variety of methods including, for example, screen printing, ink jet printing, spraying, painting, striping along a row or column of aligned and/or adjacent electrodes, and the like. Other components may be deposited separately or with the redox mediator and/or second electrode transfer agent including, for example, surfactants, polymers, polymer films, preservatives, binders, buffers, and cross-linkers.

After disposing the spacer, redox mediator, and second electron transfer agent, the substrate can be folded to form the sensor. The faces of the substrate are joined by the adhesive of the spacer. After bringing the faces together, the sensor can be cut out using a variety of methods including, for example, die cutting, slitting, or otherwise cutting away the excess substrate material and separating the individual sensors. In some embodiments, a combination of methods

Figure 20C (inverted with respect to Figures 20A and 20B) illustrates a second substrate 548 with three counter (or counter/reference) electrodes 550, 552, 554.

Figures 21A, 21B, and 21C illustrate another example of a tip-filling sensor arrangement. Figure 21A illustrates a first substrate 560 with a working electrode 562. Figure 21B illustrates a spacer 564 defining a channel 566. Figure 21C (inverted with respect to Figures 21A and 21B) illustrates a second thin film substrate 568 with two counter (or counter/reference) electrodes 570, 572. A vent hole 574 (indicated as a shaded region in Figure 21C) is provided through the second substrate. In the illustrated embodiment, this vent hole 574 is made through only the substrate 568 that carries the counter electrode(s) and, optionally, the spacer 564. In this embodiment, the vent hole may be formed by, for example, die cutting a portion of the substrate. This die cut may remove a portion of at least one counter electrode, but a sufficient amount of the counter electrode should remain for contact with the sample in the channel and for electrical connection to a contact at the other end of the sensor. In another embodiment, the vent hole 574 may be made through all of the layers or through the first substrate and not the second substrate.

Another embodiment is illustrated in Figures 22A, 22B, and 22C, with a different shape. This sensor includes a first substrate 579 with at least one working electrode 580, as illustrated in Figure 22A. The sensor also includes a spacer 581 with a channel 582 formed in the spacer 581, as shown in Figure 22B. The sensor further includes a second substrate 583 with two counter electrodes 584, 585, as shown in Figure 22C (inverted with respect to Figures 22A and 22B). A venting aperture 586 is cut typically through all of the layers and extends from a side of the sensor. In some embodiments, the venting aperture and the front portion 587 of the sensor are simultaneously cut with a reproducible distance between the venting aperture and the front portion 587 of the sensor to provide a reproducible length for the channel 582 and the working electrode 580. Figures 22A, 22B, and 22C also illustrate another feature that can be used with any sensor arrangement. An indentation 588 may be formed at the filling opening of the channel 582 to facilitate the drawing of fluid into the sensor. In this configuration, the fluid is not provided with a flat face, but rather an indented face that may aid in wicking or capillary

not formed using straight lines, but there is an expanded region 618 within the sample chamber. This permits larger sample chambers without forming larger openings. This expanded region can be formed as any shape including circular, square, rectangular, and other regular and irregular shapes.

Figure 25 is an example of an assembled sensor illustrating another alternative sensor arrangement for a side-fill sensor 620. This sensor includes extensions 622 from the sensor body 624 to indicate to a user where the openings for the sample chamber 626 are provided.

One optional feature is illustrated in Figure 32 which is an edge-on view of the sensor from the inside of the meter. Figure 32 illustrates a first substrate 1120 and a second substrate 1130 that extend into the meter from the remainder of the sensor 1100 (i.e., portion 1140 is recessed with respect to substrates 1120 and 1130 in Figure 32). Examples of this configuration are illustrated in Figures 18A-18C and 24A-24C. Typically, the sensor 1100 is coupled to a meter 1110 that includes contact pads (not shown) that contact the contact regions (e.g., regions 503, 511, and 513 in Figures 18A and 18C) of the electrodes of the sensor 1100. The end of the sensor 1100 which contains the contact regions can be slid into the meter 1110. It is typically important that the contact pads of the meter 1110 make contact with the correct contact regions of the sensor so that the working electrode and counter electrode(s) are correctly coupled to the meter. In some instances, the sensor is configured so that the contact region for the working electrode on the first substrate 1120 has a different width, w1, than width, w2, for the contact region of the second substrate 1130 carrying the counter electrode(s). Examples of electrode configurations with this structure are provided in Figures 18A-18C and 24A-24C. To ensure proper insertion of the sensor 1100 into the meter 1110, the meter 1110 may include a raised area 1140 that prevents or hinders the insertion of the sensor in an improper direction. For example, the width, w2, of the contact region of the second substrate 1130 may be wider than the width, w1, of the contact region of the first substrate 1120, as illustrated in Figure 32. In this instance, the raised area 1140 is positioned to allow sensor 1100 to be slid into the meter so that the first substrate 1120 is next to the surface 1150 from which the raised area 1140 protrudes, but

requiring the user to cock the device prior to use, the risk of inadvertently triggering the lancet is minimized.

Preferably, the lancing instrument is automatically triggered when the lancing instrument is pressed firmly against the skin with an adequate amount of pressure. As is already known in the art, a larger sample of body fluid such as blood or interstitial fluid is expressed when pressure is applied around a site where a hole has been created the skin. For example, see the above-mentioned U.S. patents to Integ and Amira as well as the tip design of the lancing instruments sold by Becton Dickenson. All of these lancing devices have a protruding ring that surrounds the lancing site to create pressure that forces sample out of the wound. However, all of these devices require the user to apply adequate pressure to the wound site to express the sample, and all of the lancing instruments are triggered by a button push by the user. Design of an appropriate pressure trigger is well-known to one skilled in the art.

Preferably, the lancing instrument will also permit the user to adjust the depth of penetration of the lancet into the skin. Such devices are already commercially available from companies such as Boehringer Mannheim and Palco. This feature allows users to adjust the lancing device for differences in skin thickness, skin durability, and pain sensitivity across different sites on the body and across different users.

In a more preferred embodiment, the lancing instrument and the test reader are integrated into a single device. To operate the device the user need only insert a disposable cartridge containing a measurement strip and lancing device into the integrated device, cock the lancing instrument, press it against the skin to activate it, and read the result of the measurement. Such an integrated lancing instrument and test reader simplifies the testing procedure for the user and minimizes the handling of body fluids.

Figure 26 illustrates another example of an integrated sample acquisition and sensor device 700. The integrated sample acquisition and sensor device 700 includes a housing 702, a skin piercing member (e.g., a lancet) 704, a piercing/collecting aperture 706, an optionally removable sensor 708, a sensor guide

One example of operation includes cocking the skin piercing member 704 and then releasing the skin piercing member 704 so that it extends out of the housing 702 through the piercing/collecting aperture 706 and pierces the skin of the user. The skin piercing element 704 optionally pushes the sensor out of the way while extending out of the housing. The skin piercing element 704 is retracted back within the housing 702 using the retraction mechanism 714. Upon retraction of the skin piercing element, the sensor collects a sample fluid from the pierced skin through an opening in the sensor 708.

If a sensor reader is used, the sensor reader may also be configured to couple with a contact end of the sensor. The sensor reader may include a potentiostat or other component to provide a potential and/or current for the electrodes of the sensor. The sensor reader may also include a processor (e.g., a microprocessor or hardware) for determining analyte concentration from the sensor signals. The sensor reader may include a display or a port for coupling a display to the sensor. The display may display the sensor signals and/or results determined from the sensor signals including, for example, analyte concentration, rate of change of analyte concentration, and/or the exceeding of a threshold analyte concentration (indicating, for example, hypo- or hyperglycemia). This sensor reader may be used in conjunction with the integrated sample acquisition and sensor device or the sensor reader may be used with the sensor alone, the contacts of the sensor making connection with contacts in the sensor reader.

Operation of the Sensor

An electrochemical sensor of the invention may be operated with or without applying a potential. In one embodiment, the electrochemical reaction occurs spontaneously and a potential need not be applied between the working and counter electrodes.

In another embodiment, a potential is applied between the working and counter electrodes. Yet the potential does not need to remain constant. The magnitude of the required potential is dependent on the redox mediator. The potential at which the electrode poises itself, or where it is poised by applying an

background charge, due, at least in part, to the shuttling of a diffusible redox mediator between the working electrode and the counter electrode. This background current can be minimized or accounted for, as described above.

As an example, one sensor of the present invention is based on the reaction of a glucose molecule with two $[Os(dmo\text{-}phen)_2(NMI)Cl]^{2+}$ cations, where dmo-phen is 4,8-dimethoxy phenanthroline and NMI is N-methyl-imidazole, in the presence of glucose oxidase to produce two $[Os(dmo\text{-}phen)_2(NMI)Cl]^{+}$ cations, two protons, and an oxidation product of glucose, for example, gluconolactone or another ketone. The amount of glucose present is assayed by electrooxidizing the $[Os(dmo\text{-}phen)_2(NMI)Cl]^{+}$ cations to $[Os(dmo\text{-}phen)_2(NMI)Cl]^{2+}$ cations and measuring the total charge passed.

Those skilled in the art will recognize that there are many different reactions that will provide the same result; namely the electrolysis of an analyte through a reaction pathway incorporating a redox mediator. Equations (1) and (2) are a non-limiting example of such a reaction.

Coulometry

In a preferred embodiment of the invention, coulometry is used to determine the concentration of the analyte. This measurement technique utilizes current measurements obtained at intervals over the course of the assay, to determine analyte concentration. These current measurements are integrated over time to obtain the amount of charge, Q, passed to or from the electrode. Q is then used to calculate the concentration of the analyte (C_A) by the following equation (when the redox mediator is non-leachable):

$$C_A = Q/nFV \qquad (3a)$$

where n is the number of electron equivalents required to electrolyze the analyte, F is Faraday's constant (approximately 96,500 coulombs per equivalent), and V is the volume of sample in the measurement zone. When using a diffusible mediator, the concentration of the analyte can be obtained from the following equation:

$$Q_{Back}/Q_G = (D_M C_M/d^2)(t\, n_M/(0.9\, n_G\, C_G)) = (D_M C_M / d^2) \times (6.7 \times 10^6)$$

For example, if the ratio of Q_{Back}/Q_G is 5, then $(D_M C_M)/ d^2$ is 7.5×10^{-7} moles/(cm^3 sec). Also for example, if the ratio of Q_{Back}/Q_G is 1, then $(D_M C_M)/ d^2$ is 1.5×10^{-7} moles/(cm^3 sec). Still another example, if the ratio is 0.1, then $(D_M C_M)/ d^2$ is 1.5×10^{-8} moles/(cm^3 sec). Thus, depending on the ratio desired, a sensor may be configured to have the desired ratio by choosing D_M, C_M, and d accordingly. For example, the concentration of the redox mediator may be reduced (i.e., C_M may be reduced). Alternatively, or additionally, the diffusion of the redox mediator may be reduced by, for example, having a barrier to the flow of the diffusible mediator to the counter electrode (i.e., reduce the effective diffusion coefficient of the redox mediator—D_M). Other sensor configurations are also suitable for controlling the ratio of background signal to signal generated by the analyte and will be described below.

The background charge, Q_{back}, can be accounted for in a variety of ways. Q_{back} can be made small, for example, by using only limited amounts of diffusible redox mediator; by providing a membrane over the counter electrode that limits diffusion of the redox mediator to the counter electrode; or by having a relatively small potential difference between the working electrode and the counter electrode. Other examples of sensor configurations and methods suitable for reducing Q_{back} include those already described such as sensors having a redox mediator reaction rate at the working electrode that is significantly faster than that at the counter electrode; immobilizing the redox mediator on the working electrode; having the redox mediator become immobilized on the counter or counter/reference electrode upon its reaction at the counter or counter/reference electrode; or slowing the diffusion of the redox mediator.

Alternatively, the sensor may be calibrated individually or by batch to determine a calibration curve or a value for Q_{back}. Another option is to include a second electrode pair that is missing an item necessary for electrolysis of the analyte, such as, for example, the second electron transfer agent, so that the entire signal from this second electrode pair corresponds to Q_{back}.

if the analyte were completely or nearly completely electrolyzed and, using equation (3a) or (3b), the concentration of the analyte is calculated.

Although coulometry has the disadvantage of requiring the volume of the measured sample be known, coulometry is a preferred technique for the analysis of the small sample because it has the advantages of, for example, no temperature dependence for the measurement, no enzyme activity dependence for the measurement, no redox-mediator activity dependence for the measurement, and no error in the measurement from depletion of analyte in the sample. As already described above, coulometry is a method for determining the amount of charge passed or projected to pass during complete or nearly complete electrolysis of the analyte. One coulometric technique involves electrolyzing the analyte on a working electrode and measuring the resulting current between the working electrode and a counter electrode at two or more times during the electrolysis. The electrolysis is complete when the current reaches a steady state. The charge used to electrolyze the sample is then calculated by integrating the measured currents over time and accounting for any background signal. Because the charge is directly related to the amount of analyte in the sample there is no temperature dependence of the measurement. In addition, the activity of the enzyme does not affect the value of the measurement, but only the time required to obtain the measurement (i.e., less active enzyme requires a longer time to achieve complete electrolysis of the sample) so that decay of the enzyme over time will not render the analyte concentration determination inaccurate. And finally, the depletion of the analyte in the sample by electrolysis is not a source of error, but rather the objective of the technique. (However, the analyte need not be completely electrolyzed if the electrolysis curve is extrapolated from the partial electrolysis curve based on well-known electrochemical principles.)

Non-Coulometric Assays

Although coulometric assays are useful, those skilled in the art will recognize that a sensor of the invention may also utilize potentiometric, amperometric, voltammetric, and other electrochemical techniques to determine the

used for coulometric measurements may be used. Examples include all of the methods and structures described above, such as performing the electrochemical assay at relatively low applied potential, electrooxidizing the analyte at negative applied potentials or electroreducing the analyte at positive applied potentials, using a counter electrode at which the redox mediator reacts relatively slowly (particularly as compared to the reaction of the redox mediator at the working electrode), and/or using a redox mediator that undergoes an irreversible reaction at the counter electrode. Other examples are discussed below.

As described for coulometric measurements, it is preferred that the sensor be designed and operated so that the background signal is at most five times the size of the signal generated by electrolysis of the analyte. Preferably, the background signal is at most 200%, 100%, 50%, 25%, 10%, or 5% of the signal generated by electrolysis of an amount of analyte. The amount of analyte against which the background signal is compared is described above in the section entitled "Background Signal." In the case of amperometry, the signal generated by electrolysis of an amount of analyte is the current at the time or times at which the measurement is taken. In the case of potentiometry, the signal generated by electrolysis of an amount of analyte is the potential at the time or times at which the measurement is taken.

Under a given set of operating conditions, for example, temperature, cell geometry, and electrode size, the magnitude of the background current, I_{back}, is given by the following expression:

$$i_{back} = KC_M D_M / d$$

where: K is a proportionality constant; C_M is the concentration of the mediator in the measurement zone; D_m is the effective diffusion coefficient of the mediator in the measurement zone under normal operating conditions; and d is the distance between the electrodes.

It is desirable to reduce background current for non-coulometric assays. The sensor configurations and methods described above are generally useful and include, for example, using low concentrations of the redox mediator and/or the second electron transfer agent (e.g., enzyme) relative to the concentration of the

In some amperometric or potentiometric embodiments, the redox mediator circulation is decreased by separating the working electrode from the counter or counter/reference electrode such that the distance through which the redox mediator would diffuse during the measurement period is no greater than, for example, the distance between the electrodes. A redox mediator can diffuse a distance equal to $(D_m t)^{1/2}$, where D_m is the effective diffusion coefficient for the medium between the electrodes and t is time. For a measurement time period of 30 seconds and a redox mediator with effective diffusion coefficient between 10^{-5} and 10^{-6} cm^2/second, the electrodes should be separated by at least 100μm, preferably at least 200μm, and even more preferably at least 400μm.

One method of separating the working and counter electrodes is to use a thicker spacer between the electrodes. One alternative method is illustrated in Figure 27. In this embodiment, the working electrode 740 is disposed on a first substrate 742 and the counter electrode 744 is disposed on a second substrate 746 (alternatively, the electrodes may be disposed on the same substrate). The working electrode 742 and the counter electrode 744 are offset so that the effective distance, d, between the two electrodes is greater than the thickness, w, of the spacer layer 748. In one embodiment, the distance between the electrodes, d, is selected to be in the range of 25 to 1000 μm, 50 to 500 μm, or 100 to 250 μm.

Additionally or alternatively, in the case of steady-state amperometry and potentiometry, background signal may be controlled by limiting the rate of electrolysis such that the rate is slow enough to prevent the analyte concentration from decreasing by more than about 20%, 10%, or 5% or less, during a measurement period, e.g., 30 second, 1 minute, 5 minutes, or 10 minutes. In some instances, to control the rate of electrolysis the concentration or activity of the second electron transfer agent may be reduced and/or the working electrode area may be reduced.

For example, the second electron transfer agent can be an enzyme and the enzyme activity can be a limiting factor for the electrolysis rate. If, for example, the analyte concentration is 5mM glucose (i.e., 5×10^{-9} moles of glucose in 1 μl) and no more than 10% of the glucose (5×10^{-10} moles) is to be electrooxidized during a 30-second measurement period, the current should not exceed 3.3×10^{-6} amperes for

While this description has described electrolysis of an analyte, one skilled in the art would recognize that the same devices and techniques would also be suitable for measurements of the average oxidation state of the mediator, such as, for example, in Cottrell types of reactions.

Air-oxidizable Redox Mediators

In a sensor having a redox mediator, a potential source of measurement error is the presence of redox mediator in an unknown mixed oxidation state (i.e., mediator not reproducibly in a known oxidation state). The charge passed when the redox mediator is electrooxidized or electroreduced at the working electrode is affected by its initial oxidation state. Referring to equations (1) and (2) discussed above under the section entitled "Operation of the Sensor," the current not attributable to the oxidation of biochemical B will flow because of electrooxidation of that portion of the redox mediator, A, that is in its reduced form prior to the addition of the sample. Thus, it may be important to know the oxidation state of the analyte prior to introduction of the sample into the sensor. Furthermore, it is desirable that all or nearly all of the redox mediator have the same state or extent of oxidation prior to the introduction of the sample into the sensor.

Each redox mediator has a reduced form or state and an oxidized form or state. It is preferred that the amount of redox mediator in the reduced form prior to the introduction of sample be significantly smaller than the expected amount of analyte in a sample in order to avoid a significant background contribution to the measured current. In this embodiment of the invention, the molar amount of redox mediator in the reduced form prior to the introduction of the analyte is preferably no more than, on a stoichiometric basis, about 10%, and more preferably no more than about 5%, and most preferably no more than 1%, of the molar amount of analyte for expected analyte concentrations. (The relative molar amounts of analyte and redox mediator are compared based on the stoichiometry of the applicable redox reaction. If, for example, two moles of redox mediator are needed to electrolyze one mole of analyte, then the molar amount of redox mediator in the reduced form prior to introduction of the analyte is preferably no more than 20% and more preferably no

necessary that the mediator be completely oxidized to the higher-valent state. Additionally, it is desirable that the air oxidation of the dissolved redox mediator should not be so fast that air-oxidation during the assay can interfere with or introduce error into the measurements.

Suitable mediators which are both air-oxidizable (i.e., O_2-oxidizable) and have electron transfer capabilities have been described hereinabove. One particular family of useful mediators are osmium complexes which are bound to electron-rich nitrogen-containing heterocycles or a combination of electron-rich nitrogen-containing heterocycles and halides. Electron-rich nitrogen-containing heterocycles include, but are not limited to, imidazole derivatives and pyridine or phenanthroline derivatives that contain electron-donating substituents such as alkyl, alkoxy, amino, alkylamino, amido and mercapto groups. Preferably, the osmium complexes have no more than one halide coordinated to the metal, so that the mediators are overall positively charged and thus are water soluble. An example is osmium complexed with mono-, di-, and polyalkoxy-2,2'-bipyridine. Other examples include mono-, di-, and polyalkoxy-1,10-phenanthroline, where the alkoxy groups have a carbon to oxygen ratio sufficient to retain solubility in water, are air-oxidizable. These osmium complexes typically have two substituted bipyridine or substituted phenanthroline ligands, the two ligands not necessarily being identical. These osmium complexes are further complexed with a monomeric or polymeric ligand with one or more nitrogen-containing heterocycles, such as pyridine and imidazole. Preferred polymeric ligands include poly(4-vinyl pyridine) and, more preferably, poly(1-vinyl imidazole) or copolymers thereof. $[Os[4,4'\text{-dimethoxy-}2,2'\text{-bipyridine}]_2Cl]^{+/+2}$ complexed with a poly(1-vinyl imidazole) or poly(4-vinyl pyridine) has been shown to be particularly useful as the Os^{+2} cation is oxidizable by O_2 to Os^{+3}. Similar results are expected for complexes of $[Os(4,7\text{-dimethoxy-}1,10\text{-phenanthroline})_2Cl]^{+/+2}$,and other mono-, di-, and polyalkoxy bipyridines and phenanthrolines, with the same polymers. Other halogen groups such as bromine may be substituted for chlorine. Similar results are also expected for complexes comprising the following structures, as specified above:

oxidation of the redox mediator by the oxidizer is slow compared to the electrooxidation of the mediator by the electrode.

Alternatively, if the redox mediator is to be oxidized in the presence of the analyte and electroreduced at the electrode, a reducer rather than an oxidizer would be required. The same considerations for the appropriate choice of reducer and mediator apply as described hereinabove for the oxidizer.

The use of stable air-oxidizable redox mediators in the electrochemical sensors of the invention provides an additional advantage during storage and packaging. Sensors of the invention which include air-oxidizable redox mediators can be packaged in an atmosphere containing molecular oxygen and stored for long periods of time, e.g., greater than one month, while maintaining at least 80% and preferably at least 90% of the redox species in the oxidized state.

Use of the Air-Oxidizable Mediators in Optical Sensors

The air-oxidizable redox species of the present invention can be used in other types of sensors. The osmium complexes described hereinabove are suitable for use in optical sensors, due to the difference in the absorption spectra, luminescence and/or fluorescence characteristics of the complexed Os^{+2} and Os^{+3} species. Absorption, transmission, reflection, luminescence and/or fluorescence measurements of the redox species will correlate with the amount of analyte in the sample (after reaction between an analyte and the redox species, either directly, or via a second electron transfer agent such as an enzyme). In this configuration, the molar amount of redox mediator should be greater, on a stoichiometric basis, than the molar amount of analyte reasonably expected to fill the measurement zone of the sensor.

Standard optical sensors, including light-guiding optical fiber sensors, and measurement techniques can be adapted for use with the air-oxidizable mediators. For example, the optical sensors of the invention may include a light-transmitting or light reflecting support on which the air-oxidizable redox species, and preferably an analyte-responsive enzyme, is coated to form a film. The support film forms one boundary for the measurement zone in which the sample is placed.

such as the standard deviation of the averaged measurements. The average may then be recalculated while omitting the rejected values. Furthermore, subsequent readings from an electrode that produced a rejected value may be ignored in later tests if it is assumed that the particular electrode is faulty. Alternatively, a particular electrode may be rejected only after having a predetermined number of readings rejected based on the readings from the other electrodes.

In addition to using multiple electrode sensors to increase precision, multiple measurements may be made at each electrode and averaged together to increase precision. This technique may also be used with a single electrode sensor to increase precision.

Errors in assays may occur when mass produced sensors are used because of variations in the volume of the measurement zone of the sensors. Two of the three dimensions of the measurement zone, the length and the width, are usually relatively large, between about 1-5 mm. Electrodes of such dimensions can be readily produced with a variance of 2% or less. The submicroliter measurement zone volume requires, however, that the third dimension be smaller than the length or width by one or two order of magnitude. As mentioned hereinabove, the thickness of the sample chamber is typically between about 50 and about 200 μm. Manufacturing variances in the thickness may be on the order of 20 to 50 μm. Therefore, it may be desirable that a method be provided to accommodate for this uncertainty in the volume of sample within the measurement zone.

In one embodiment of the invention, depicted in Figure 5, multiple working electrodes 42, 44, 46 are provided on a base material 48. These electrodes are covered by another base, not shown, which has counter electrodes, not shown, disposed upon it to provide multiple facing electrode pairs. The variance in the separation distance between the working electrode and the counter electrode among the electrode pairs on a given sensor is significantly reduced, because the working electrodes and counter electrodes are each provided on a single base with the same spacer 28 between each electrode pair (see Figure 3).

One example of a multiple electrode sensor that can be used to accurately determine the volume of the measurement zones of the electrode pairs and

caused by a diffusible redox mediator. This procedure also reduces the error associated with other electrolyzed interferents, such as ascorbate, urate, and acetaminophen, as well as errors associated with capacitive charging and faradaic currents.

The thickness of the sample chamber can be determined by measuring the capacitance, preferably in the absence of any fluid, between electrode 46 (or any of the other electrodes 42, 44 in the absence of sorbent material) and its corresponding counter electrode. The capacitance of an electrode pair depends on the surface area of the electrodes, the interelectrode spacing, and the dielectric constant of the material between the plates. The dielectric constant of air is unity which typically means that the capacitance of this electrode configuration is a few picofarads (or about 100-1000 picofarads if there is fluid between the electrode and counter electrode given that the dielectric constant for most biological fluids is approximately 75). Thus, since the surface area of the electrodes are known, measurement of the capacitance of the electrode pair allows for the determination of the thickness of the measurement zone to within about 1-5%.

The amount of void volume in the sorbent material, can be determined by measuring the capacitance between electrode 44 (which has no second electron transfer agent) and its associated counter electrode, both before and after fluid is added. Upon adding fluid, the capacitance increases markedly since the fluid has a much larger dielectric constant. Measuring the capacitance both with and without fluid allows the determination of the spacing between the electrodes and the void volume in the sorbent, and thus the volume of the fluid in the reaction zone.

Other electrode configurations can also use these techniques (i.e., capacitance measurements and coulometric measurements in the absence of a critical component) to reduce background noise and error due to interferents and imprecise knowledge of the volume of the interrogated sample. Protocols involving one or more electrode pairs and one or more of the measurements described above can be developed and are within the scope of the invention. For example, only one electrode pair is needed for the capacitance measurements, however, additional electrode pairs may be used for convenience.

particular value to determine that the sensor has filled. Typically, the indicator electrode is further downstream from a sample inlet port than the working electrode and counter electrode.

For side-fill sensors, such as those illustrated in Figures 19A-19C and 20A-20C, two indicator electrodes may be disposed on either side of the primary counter electrode. This permits the user to fill the sample chamber from either the left or right side with an indicator electrode disposed further upstream. This three-electrode configuration is not necessary. Side-fill sensors can also have a single indicator electrode and, preferably, some indication as to which side should be placed in contact with the sample fluid.

In one embodiment, the use of three counter/reference electrodes and/or indicator electrodes, detects when the sample chamber begins to fill and when the sample chamber has been filled to prevent partial filling of the sample chamber. In this embodiment, the two indicator electrodes are held at a different potential than the largest counter/reference electrode. The start and completion of filling of the sample chamber is indicated by the flow of current between the indicator and counter/reference electrodes.

In other instances, the potential of each of the counter/reference electrodes may be the same. When the potential at all three counter/reference electrodes is the same for example, 0 volts, then as the measurement zone begins to fill, the fluid allows for electrical contact between a working electrode and the first counter/reference electrode, causing a current at the first counter/reference electrode due to the reaction of the analyte with the enzyme and the mediator. When the fluid reaches the third counter/reference electrode, another current may be measured similar to the first counter/reference electrode indicating that the measurement zone is full. When the measurement zone is full, the three counter/reference electrodes may be shorted together or their signals may be added or otherwise combined.

The indicator electrode may also be used to improve the precision of the analyte measurements according to the methods described above for multiple electrode sensors. The indicator electrode may operate as a working electrode or as a counter electrode or counter/reference electrode. In the embodiment of Figures

some instances, the sample may be heated up to 5 to 20°C above an initial temperature. In other instances, the temperature of the sample may not be known but a constant amount of power or current may be applied to the wire or ink.

EXAMPLES

The invention will be further characterized by the following examples. These examples are not meant to limit the scope of the invention which has been fully set forth in the foregoing description. Variations within the concepts of the invention are apparent to those skilled in the art.

Example 1
Preparation of a Small Volume *in vitro* Sensor for the Determination of Glucose Concentration

A sensor was constructed corresponding to the embodiment of the invention depicted in Figure 1. The working electrode was constructed on a Mylar™ film (DuPont), the Mylar™ film having a thickness of 0.175 mm and a diameter of about 2.5 cm. An approximately 12 micron thick carbon pad having a diameter of about 1 cm was screen printed on the Mylar™ film. The carbon electrode was overlaid with a water-insoluble dielectric insulator (Insulayer) having a thickness of 12 μm, and a 4 mm diameter opening in the center.

The center of the carbon electrode, which was not covered by the dielectric, was coated with a non-leachable redox mediator. The redox mediator was formed by complexing poly(1-vinyl imidazole) with Os(4,4'-dimethoxy-2,2'-bipyridine)$_2$Cl$_2$ followed by cross-linking glucose oxidase with the osmium polymer using polyethylene glycol diglycidyl ether (PEGDGE) as described in Taylor et al., *J. Electroanal. Chem.*, 396:511 (1995). The ratio of osmium to imidazole functionalities in the redox mediator was approximately 1:15. The mediator was deposited on the working electrode in a layer having a thickness of 0.6 μm and a diameter of 4 mm. The coverage of the mediator on the electrode was about 60 μ g/cm^2 (dry weight). A spacer material was placed on the electrode surrounding the

wicked into the sorbent when contact was made between the sample and the sorbent tab. As the sample chamber filled and the sample made contact with the electrodes, current flowed between the electrodes. When glucose molecules in the sample came in contact with the glucose oxidase on the working electrode, the glucose molecules were electrooxidized to gluconolactone. The osmium redox centers in the redox mediator then reoxidized the glucose oxidase. The osmium centers were in turn reoxidized by reaction with the working electrode. This provided a current which was measured and simultaneously integrated by a coulometer (EG&G Princeton Applied Research Model #173).

The electrochemical reaction continued until the current reached a steady state value which indicated that greater than 95% of the glucose had been electroreduced. The current curve obtained by measurement of the current at specific intervals was integrated to determine the amount of charge passed during the electrochemical reaction. These charges were then plotted versus the known glucose concentration to produce a calibration curve.

The sensor was tested using 0.5 μL aliquots of solutions containing known concentrations of glucose in a buffer of artificial cerebrospinal fluid or in a control serum (Baxter-Dade, Monitrol Level 1, Miami, FL) in the range of 3 to 20 mM glucose. The artificial cerebrospinal fluid was prepared as a mixture of the following salts: 126 mM NaCl, 27.5 mM $NaHCO_3$, 2.4 mM KCl, 0.5 mM KH_2PO_4, 1.1 mM $CaCl_2 \cdot 2H_2O$, and 0.5 mM Na_2SO_4.

The results of the analyses are shown in Table 1 and in Figure 7. In Table 1, Q_{avg} is the average charge used to electrolyze the glucose in 3-6 identical test samples (Figure 7 graphs the charge for each of the test samples) and the 90% rise time corresponds to the amount of time required for 90% of the glucose to be electrolyzed. The data show a sensor precision of 10- 20%, indicating adequate sensitivity of the sensor for low glucose concentrations, as well as in the physiologically relevant range (30 μg/dL - 600 μg/dL).

There were 34 data points. Of those data points 91% fell in zone A, 6% in zone B, and 3 % in zone C. Only one reading was determined to be in zone C. This reading was off-scale and is not shown in figure 9. Thus, 97% of the readings fell in the clinically acceptable zones A and B.

The total number of Os atoms was determined by reducing all of the Os and then electrooxidizing it with a glucose-free buffer in the sample chamber. This resulted in a charge of 59.6 ± 5.4 μC. Comparison of this result with the glucose-free buffer result in Table 1 indicated that less than 20% of the Os is in the reduced form prior to introduction of the sample. The variability in the quantity of osmium in the reduced state is less than 5% of the total quantity of osmium present.

Example 2
Response of the Glucose Sensor to Interferents

A sensor constructed in the same manner as described above for Example 1 was used to determine the sensor's response to interferents. The primary electrochemical interferents for blood glucose measurements are ascorbate, acetaminophen, and urate. The normal physiological or therapeutic (in the case of acetaminophen) concentration ranges of these common interferents are:

ascorbate: 0.034 - 0.114 mM

acetaminophen: 0.066 - 0.200 mM

urate (adult male): 0.27 - 0.47 mM

Tietz, in: *Textbook of Clinical Chemistry*, C.A. Burtis and E.R. Ashwood, eds., W.B. Saunders Co., Philadelphia 1994, pp. 2210-12.

Buffered glucose-free interferent solutions were tested with concentrations of the interferents at the high end of the physiological or therapeutic ranges listed above. The injected sample volume in each case was 0.5 μL. A potential of +100 mV or +200 mV was applied between the electrodes. The average charge (Q_{avg}) was calculated by subtracting an average background current obtained from a buffer-only (i.e., interferent-free) solution from an average signal recorded with interferents present. The resulting average charge was compared with the

Example 3

Sensor with Glucose Dehydrogenase

A sensor similar to that described for Example 1 was prepared and used for this example, except that glucose oxidase was replaced by pyrroloquinoline quinone glucose dehydrogenase and a potential of only +100 mV was applied as opposed to the +200 mV potential in Example 1. The results are presented in Table 3 below and graphed in Fig. 10.

TABLE 3
Sensor Results Using Glucose Dehydrogenase

	n	Q_{avg} (TC)	90% rise time (s)
buffer	4	21.7 ± 5.2	14 ± 3
3 mM glucose/buffer	4	96.9 ± 15.0	24 ± 6
6 mM glucose/buffer	4	190.6 ± 18.4	26 ± 6
10 mM glucose/buffer	4	327.8 ± 69.3	42 ± 9

The results indicated that the charge obtained from the glucose dehydrogenase sensor was much larger than for the comparable glucose oxidase sensor, especially for low concentrations of glucose. For 4 mM glucose concentrations the measurements obtained by the two sensors differed by a factor of five. In addition, the glucose dehydrogenase sensor operated at a lower potential, thereby reducing the effects of interferent reactions.

In addition, the results from Table 3 were all fit by a linear calibration curve as opposed to the results in Example 1, as shown in Fig. 10. A single linear calibration curve is greatly preferred to simplify sensor construction and operation.

Also, assuming that the interferent results from Table 2 are applicable for this sensor, all of the interferents would introduce an error of less than 7% for a 3 mM glucose solution at a potential of 100 mV.

indicated by the achievement of a stabilized, steady-state current. The total charge, Q, required for lactate electrooxidation was found by integration of the differential current registered from the flow stoppage until the current reached a steady-state. The concentration was then calculated by the following equation:

$$[\text{lactate}] = Q/2FV \quad (4)$$

where V is the volume of sample within the measurement zone and F is Faraday's constant.

This assay was performed using lactate solutions having nominal lactate concentrations of 1.0, 5.0, and 10.0 mM. The measured concentrations for the assay were 1.9, 5.4, and 8.9 mM respectively.

Example 5
Determination of the Oxidation State of Os(4,4'-dimethoxy-2,2'-bipyridine)$_2$Cl$^{+/+2}$ Complexed with poly(1-vinyl imidazole)

A sensor having a three electrode design was commercially obtained from Ecossensors Ltd., Long Hanborough, England, under the model name "large area disposable electrode". The sensor contained parallel and coplanar working, reference and counter electrodes. The working surface area (0.2 cm^2) and counter electrodes were formed of printed carbon and the reference electrode was formed of printed Ag/AgCl. A redox mediator was coated on the carbon working electrode. The redox mediator was formed by complexation of poly(1-vinyl imidazole) with Os(4,4'-dimethoxy-2,2'-bipyridine)$_2$Cl$_2$ in a ratio of 15 imidazole groups per Os cation followed by cross linking the osmium polymer with glucose oxidase using polyethylene glycol diglycidyl ether.

The electrode was cured at room temperature for 24 hours. The coplanar electrode array was then immersed in a buffered electrolyte solution, and a potential of +200 mV (sufficient for conversion of Os(II) to Os(III),) was applied between the working electrode and the reference electrode.

Upon application of the potential, an undetectable charge of less than 1 μC was passed. Subsequent reduction and reoxidation of the redox mediator

adapted to transmit light through the assembled sensor to an optical density detector or to a luminescence and/or fluorescence detector. As sample fills the sample chamber and the redox mediator is oxidized, changes in the absorption, transmission, reflection or luminescence and/or fluorescence of the redox mediator in the chamber are correlated to the amount of glucose in the sample.

Example 8
Blood Volumes from Upper Arm Lancet Sticks

The forearm of a single individual was pierced with a lancet multiple times in order to determine the reproducibility of blood volumes obtained by this method. Despite more than thirty lancet sticks in the anterior portion of each forearm and the dorsal region of the left forearm, the individual identified each stick as virtually painless.

The forearm was pierced with a Payless Color Lancet. The blood from each stick was collected using a 1 μL capillary tube, and the volume was determined by measuring the length of the blood column. The volumes obtained from each stick are shown below in Table 4.

Example 10
Measuring Glucose using Sensor with Diffusible Redox Mediat r at a Potential of 0 V.

Sensors were formed as described in Example 9 and used to measure glucose/buffer solutions at 0, 90, 180, 270, and 360 mg/dL glucose concentration. The charge measured over time for each of these solutions is graphed in Figure 15. In the absence of glucose, the sensor indicates about 3 mg/dL glucose concentration. Figure 16 illustrates the measured charge versus glucose concentration for three sensors at each glucose concentration. The measured charge varies linearly with glucose concentration similar to what is observed for sensors using non-leachable redox mediator.

Example 11
Other Sensors Formed Using Diffusible Redox Mediator

Sensors A and B were formed by printing graphite ink (Graphite #G4491, Ercon, Wareham, MA) on a polyester substrate. For Sensor A, a mixture of 8.0 $\mu g/cm^2$ [Os(dimethyoxybipyridine)$_2$(vinyl imidazole)Cl]Cl, 34.7 $\mu g/cm^2$ PQQ-glucose dehydrogenase, and 26.6 $\mu g/cm^2$ Zonyl FSO® surfactant (E.I. duPont de Nemours & Co., Inc., Wilmington, DE) were deposited on a portion of the working electrode. For Sensor B, a mixture of 24 $\mu g/cm^2$ [Os(dimethyoxybipyridine)$_2$(vinyl imidazole)Cl]Cl, 104 $\mu g/cm^2$ PQQ-glucose dehydrogenase, and 80 $\mu g/cm^2$ Zonyl FSO® surfactant (E.I. duPont de Nemours & Co., Inc., Wilmington, DE) were deposited on a portion of the working electrode. A 200 μm pressure sensitive adhesive tape was then formed over the working electrode of each sensor leaving only a portion of the working electrode exposed to form a sample chamber. A second polyester film with a counter electrode disposed on the film was provided over the pressure sensitive adhesive tape. The counter electrode of each sensor was formed by disposing Ag/AgCl ink (Silver/Silver Chloride #R414, Ercon, Wareham, MA) over the second polyester film. The Ag/AgCl counter electrode was coated

sample chamber was full. The sensors were laminated by three passes with a hand roller and aged for three days at room temperature over $CaSO_4$.

The sensors were constructed so that when sufficient current flowed between indicator and counter/reference electrodes, an external circuit emitted a visual signal indicating that the channel overlying the working electrode was full of blood.

A few days prior to using the sensors, dry capacitance measurements were taken to determine the uniformity of the sample chamber volume. The variation in capacitance reflected misalignment of electrodes and/or variation in adhesive thickness. The mean capacitance measured was 7.49 pF with a standard deviation of 0.28 pF or 3.8%. The maximum capacitance measured was 8.15 pF and the minimum capacitance measured was 6.66 pF.

The sensors were used to determine the glucose concentration in blood samples obtained from 23 people. In the study, the people ranged from 26 to 76 years of age, fourteen were men, and nine were women. Six of the people were diagnosed with Type 1 diabetes, sixteen were diagnosed with Type 2 diabetes, and one person was unknown regarding diabetic status. The people studied had an average hematocrit of 40.7% with a standard deviation of 3.9%. The maximum hematocrit was 49% and the minimum hematocrit was 33.2%.

One blood sample for each person was collected by pricking the finger of the subject. A small volume sensor was filled with this residual blood.

Three blood samples for each person were then collected in small volume sensors by using a 2mm Carelet™ to lance the arm. If an adequate sample was not obtained in 10 seconds, the area around the puncture wound was kneaded, and then the sensor was filled. Sixteen of the sixty-nine samples required that the wound be kneaded.

Three blood samples per person were collected by venipuncture. YSI blood glucose measurements and hematocrit measurements were taken on at least one sample. Forty-six small volume sensors were also filled with blood from these samples.

WE CLAIM:

1. A sensor for determining the concentration of an analyte in a sample fluid, the sensor comprising:

an electrode pair comprising a working electrode and a counter electrode, wherein at least a portion of the working electrode is within a distance of no more than 200 μm of a portion of the counter electrode and, optionally, the counter electrode is a counter/reference electrode;

an optional reference electrode;

a sample chamber for holding the sample fluid in electrolytic contact with the working electrode, the counter electrode, and the reference electrode, if present , the sample chamber comprising a measurement zone positioned adjacent to the working electrode, the counter electrode, and the reference electrode, if present, wherein the measurement zone is sized to contain a volume of no more than about 1 μL of sample fluid and, optionally, the sample chamber is sized to contain no more than about 1 μL of sample fluid; and

an analyte-responsive enzyme and a diffusible redox mediator disposed in the measurement zone;

wherein the sensor is configured and arranged so that a background signal generated by the diffusible redox mediator is no more than five times a signal generated by oxidation or reduction of an average normal physiological amount of analyte.

2. A sensor for determining the concentration of an analyte in a sample fluid, the sensor comprising:

an electrode pair comprising a working electrode and a counter electrode, wherein at least a portion of the working electrode is within a distance of no more than 200 μm of a portion of the counter electrode and, optionally, the counter electrode is a counter/reference electrode;

an optional reference electrode;

a sample chamber for holding the sample fluid in electrolytic contact with the

electrode, the counter electrode, and the reference electrode, if present, wherein the measurement zone is sized to contain a volume of no more than about 1 μL of sample fluid and, optionally, the sample chamber is sized to contain no more than about 1 μL of sample fluid; and

an analyte-responsive enzyme and a diffusible redox mediator disposed in the measurement zone;

wherein the analyte is glucose and the sensor is configured and arranged so that a background signal generated by the diffusible redox mediator is no more than five times a signal generated by oxidation or reduction of 5 mM glucose.

5. A sensor according to any of claims 1 to 4, wherein the sensor comprises

(a) a first substrate having a proximal end and a distal end, the first substrate defining a first side edge and a second side edge of the electrochemical sensor extending from the proximal end to the distal end of the first substrate, the distal end being configured and arranged for insertion into a sensor reader;

(b) a second substrate disposed over the first substrate, the working electrode being disposed on one of the first and second substrates and the counter electrode being disposed on one of the first and second substrates;

(c) a spacer disposed between the first and second substrates and defining a first aperture along the first side edge of the sensor and a second aperture along the second side edge of the sensor, the sample chamber extending from the first aperture to the second aperture; and

(d) at least one indicator electrode disposed on at least one of the first and second substrates and positioned relative to the sample chamber to determine when the sample chamber contains sample.

6. A sensor according to any of claims 1 to 4, wherein the sensor comprises

(a) a first substrate having a proximal end and a distal end, the distal end being configured and arranged for insertion into a sensor reader, the

11. A sensor according to any of claims 1 to 10, wherein the sensor comprises at least two indicator electrodes disposed in the sensor, wherein a first indicator electrode indicates when the measurement zone is beginning to fill with sample and a second indicator electrode indicates when the measurement zone is substantially filled with sample.

12. A sensor according to any of claims 1 to 11, wherein the sensor comprises at least two indicator electrodes disposed in the sensor, wherein two of the indicator electrodes comprise a first counter/indicator electrode and a second counter/indicator electrode with the counter electrode disposed between the first and second counter/indicator electrodes.

13. A sensor according to any of claims 1 to 12, wherein the measurement zone and the sample chamber have a same volume.

14. A sensor according to any of claims 1 to 3 and 5 to 13, wherein the analyte is glucose and the analyte-responsive enzyme is a glucose-responsive enzyme.

15. A sensor according to any of claims 1 to 13, wherein the analyte is a drug.

16. A sensor according to any of claims 1 to 15, wherein the measurement zone is bounded on at least two sides by the working electrode and the counter electrode and, optionally, the working electrode and counter electrode form a facing electrode pair with the measurement zone positioned between the working electrode and the counter electrode.

17. A sensor according to any of claims 1 to 16, wherein the sensor is configured and arranged so that the mediator oxidizes the analyte and the half-wave potential of the redox mediator, as measured by cyclic voltammetry in 0.1 M NaCl at pH 7, is no more than about +100 millivolts relative to the potential of the counter/reference electrode.

24. A sensor according to any of claims 1 to 23, wherein the working electrode has a surface area of no more than about $0.01 cm^2$ exposed in the measurement zone.

25. A sensor according to any of claims 1 to 24, wherein the activity of the enzyme is no more than 1 unit/cm^3.

26. A sensor according to any of claims 1 to 25, wherein the sensor is configured and arranged so that the diffusible redox mediator precipitates when reacted at the counter electrode.

27. A sensor according to any of claims 1 to 26, wherein the sensor is configured and arranged so that a mathematical product of the effective diffusion coefficient of the redox mediator and the concentration of the redox mediator is no more than 1 x 10^{-12} moles cm^{-1} sec^{-1} when sample fluid fills the measurement zone.

28. A sensor according to any of claims 1 to 27, wherein the diffusible redox mediator is disposed on the working electrode.

29. A sensor according to any of claims 1 to 28, wherein the analyte-responsive enzyme is disposed on the working electrode.

30. A sensor for determining a concentration of an analyte in a sample, the sensor comprising:

(a) a first substrate having a proximal end and a distal end, the first substrate defining a first side edge and a second side edge of the electrochemical sensor extending from the proximal end to the distal end of the first substrate, the distal end being configured and arranged for insertion into a sensor reader;

(b) a second substrate disposed over the first substrate;

(c) a spacer disposed between the first and second substrates and defining a first aperture along the first side edge of the sensor, a

beginning to fill with sample and a second indicator electrode indicates when the measurement zone is filled with sample.

33. A sensor according to any of claims 31 and 32, further comprising

a first substrate upon which the working electrode is disposed;

a second substrate upon which the counter electrode is disposed;

a working electrode contact pad disposed on the first substrate and electrically coupled to the working electrode;

a counter electrode contact pad disposed on the second substrate and electrically coupled to the counter electrode; and

an indicator electrode contact pad electrically coupled to the indicator electrode, the indicator electrode and the indicator electrode contact pad being disposed on a one of the first and second substrates, the indicator electrode contact pad being disposed proximate to a one of the working electrode contact pad and the counter electrode contact pad;

wherein the working electrode contact pad, the counter electrode contact pad, and the indicator electrode contact pad are exposed for electrical connection to a meter by cutout regions defined by the first and second substrates.

34. A sensor according to any of claims 30 to 33, wherein the sensor comprises at least two indicator electrodes disposed in the sensor, wherein two of the indicator electrodes comprise a first counter/indicator electrode and a second counter/indicator electrode with the counter electrode disposed between the first and second counter/indicator electrodes.

35. A method for determining a concentration of an analyte in a sample, comprising the steps of:

contacting a sample with any of the electrochemical sensors of claims 1 to 34;

generating a sensor signal at the working electrode, wherein a background signal that is generated by the diffusible redox mediator is no more than five times a

to electrolyze the analyte in the sample;

generating an analyte-responsive signal from the sensor in response to electrolysis of the analyte in the sample; and

determining the concentration of the analyte using the analyte-responsive signal.

41. A method of manufacturing any of the electrochemical sensors of claims 1 to 34, the method comprising:

(a) forming a plurality of working electrodes on a first substrate;

(b) forming a plurality of counter electrodes on a second substrate;

(c) disposing a spacer layer on one of the first and second substrates;

(d) removing a portion of the spacer layer to define sample chamber regions;

(e) laminating the first and second substrates together; and

(f) separating a plurality of electrochemical sensors from the laminated substrates, each electrochemical sensor comprising at least one of the working electrodes, at least one of the counter electrodes, and at least one of the sample chamber regions.

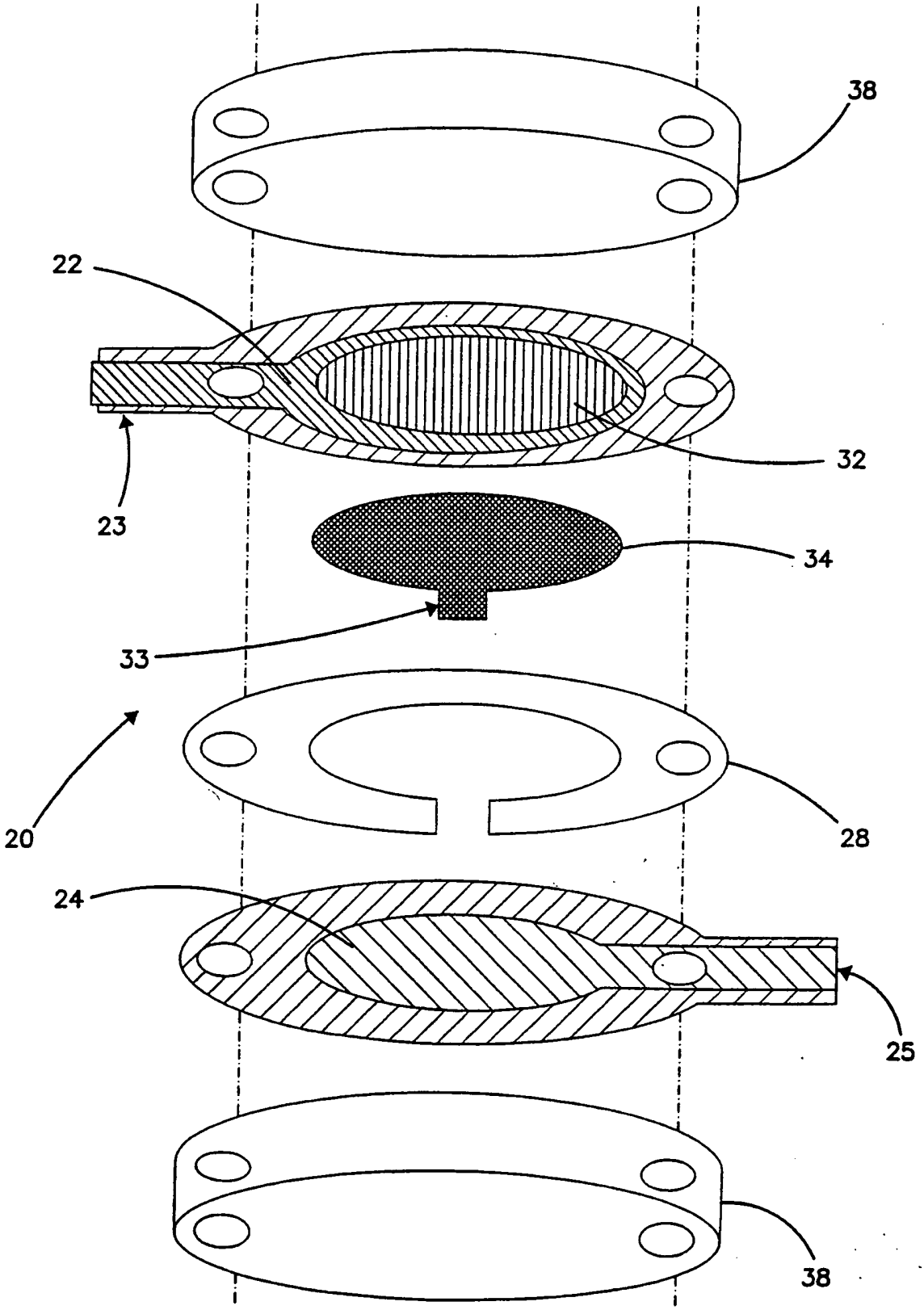
42. A method according to claim 41, wherein the first substrate is a first region of a substrate and the second substrate is a second region of the substrate and further comprising

folding the substrate to overlay the first and second regions of the substrate.

43. A method according to any of claims 41 and 42, wherein separating the plurality of electrochemical sensors comprises cutting the first and second substrates to separate the electrochemical sensors and to define the at least one end of the sample chamber of the electrochemical sensors.

44. A method according to any of claims 41 to 43, further comprising forming a plurality of indicator electrodes on one of the first and second substrates.

FIG. 1



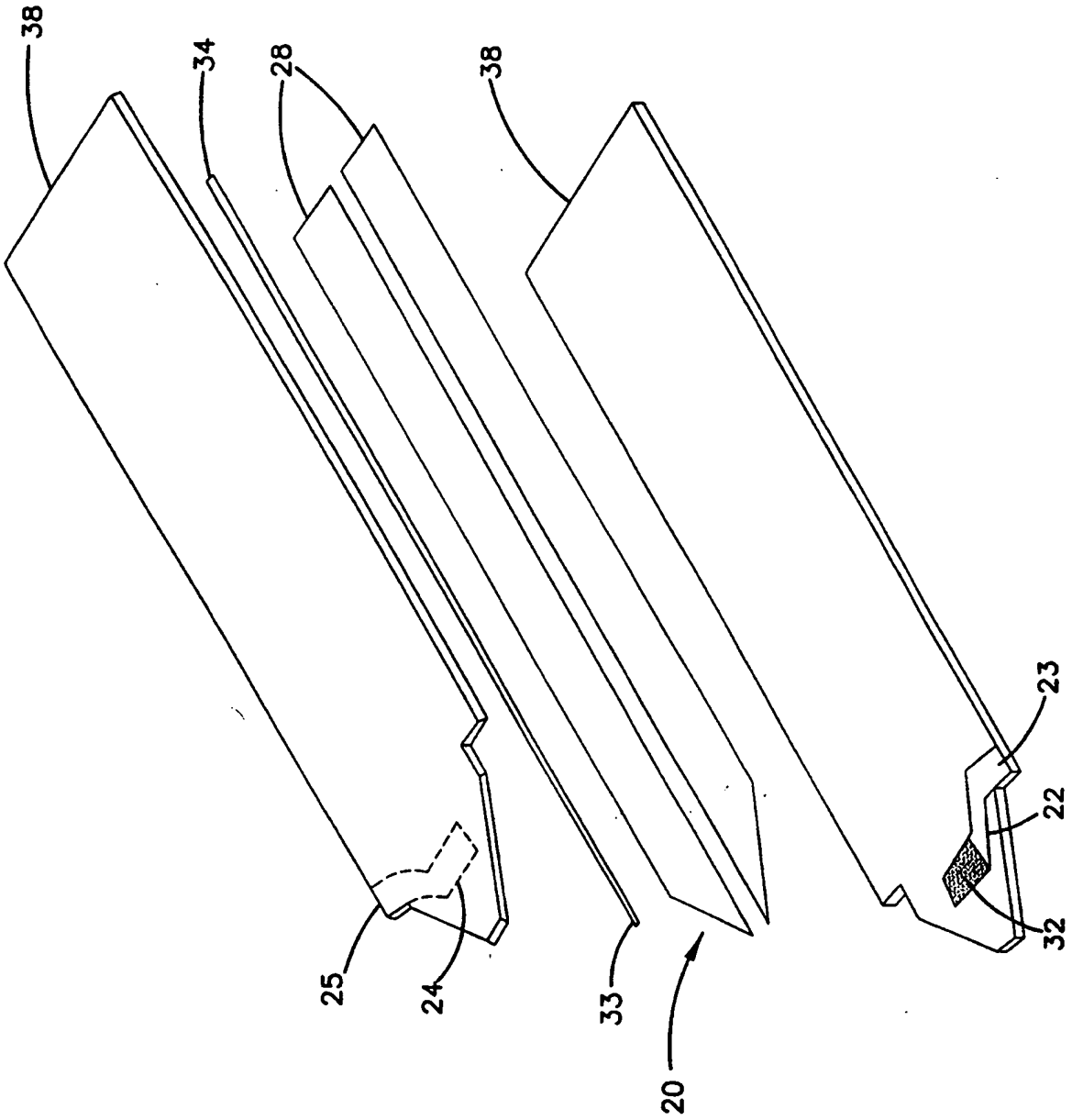


FIG. 3

FIG. 5

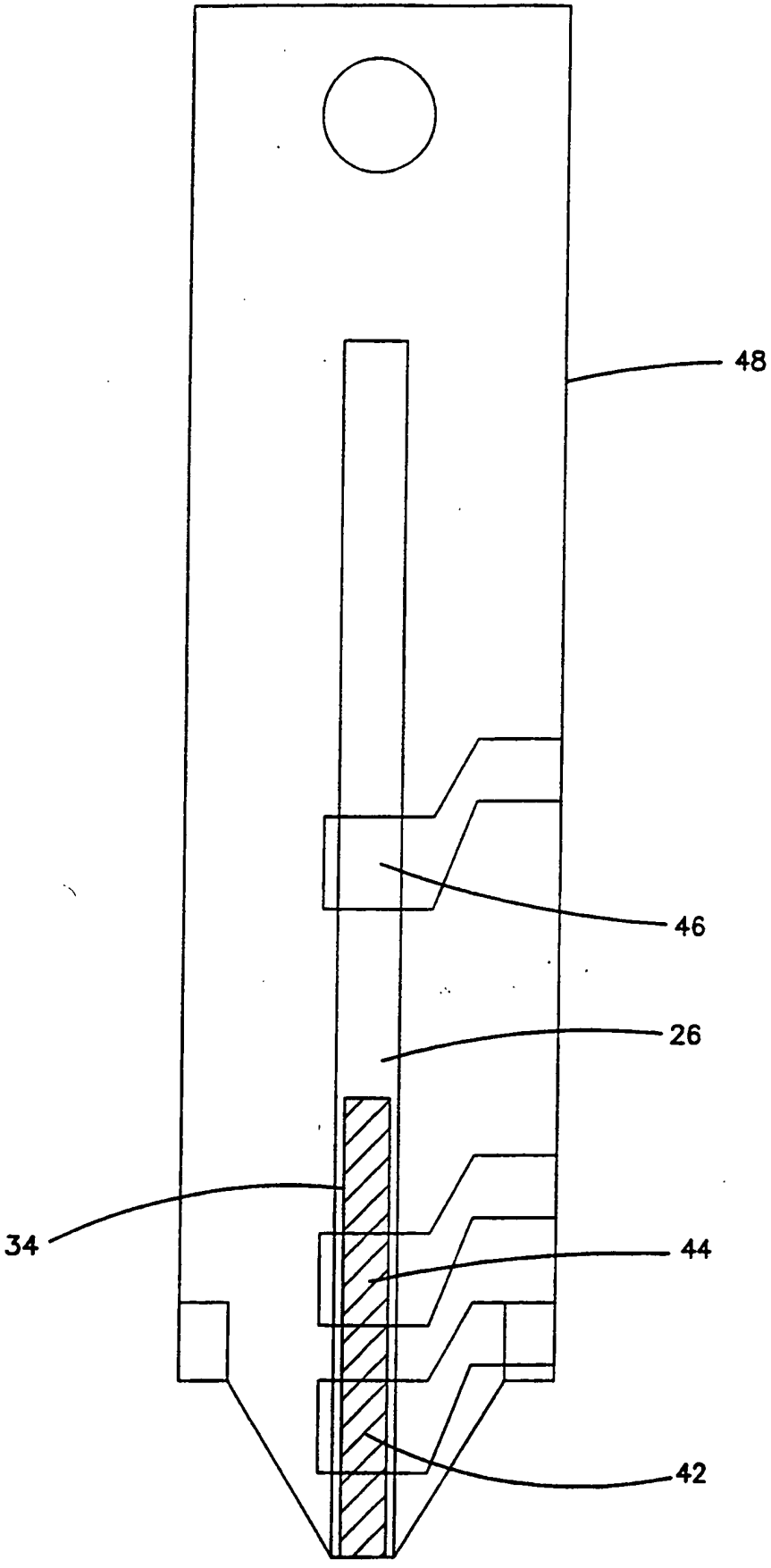
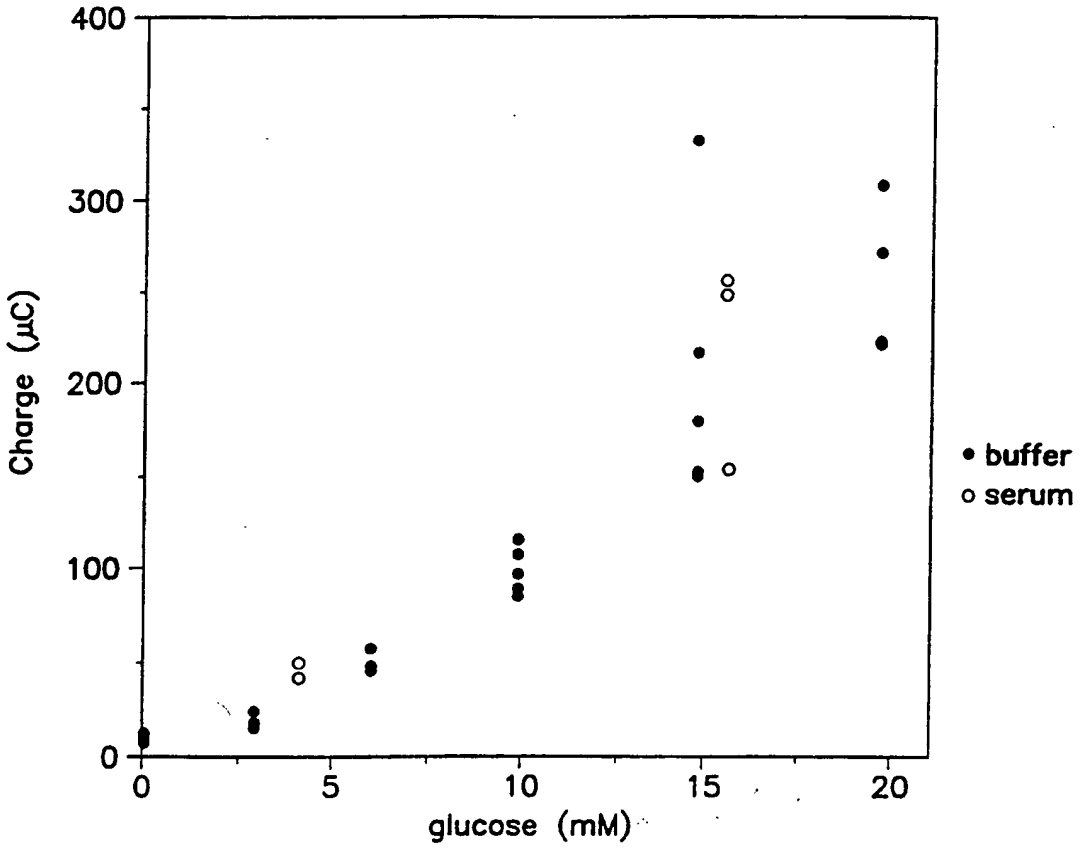


FIG. 7


400
300
200
100
0
Charge (μC)
0
5
10
15
20
glucose (mM)
• buffer
o serum

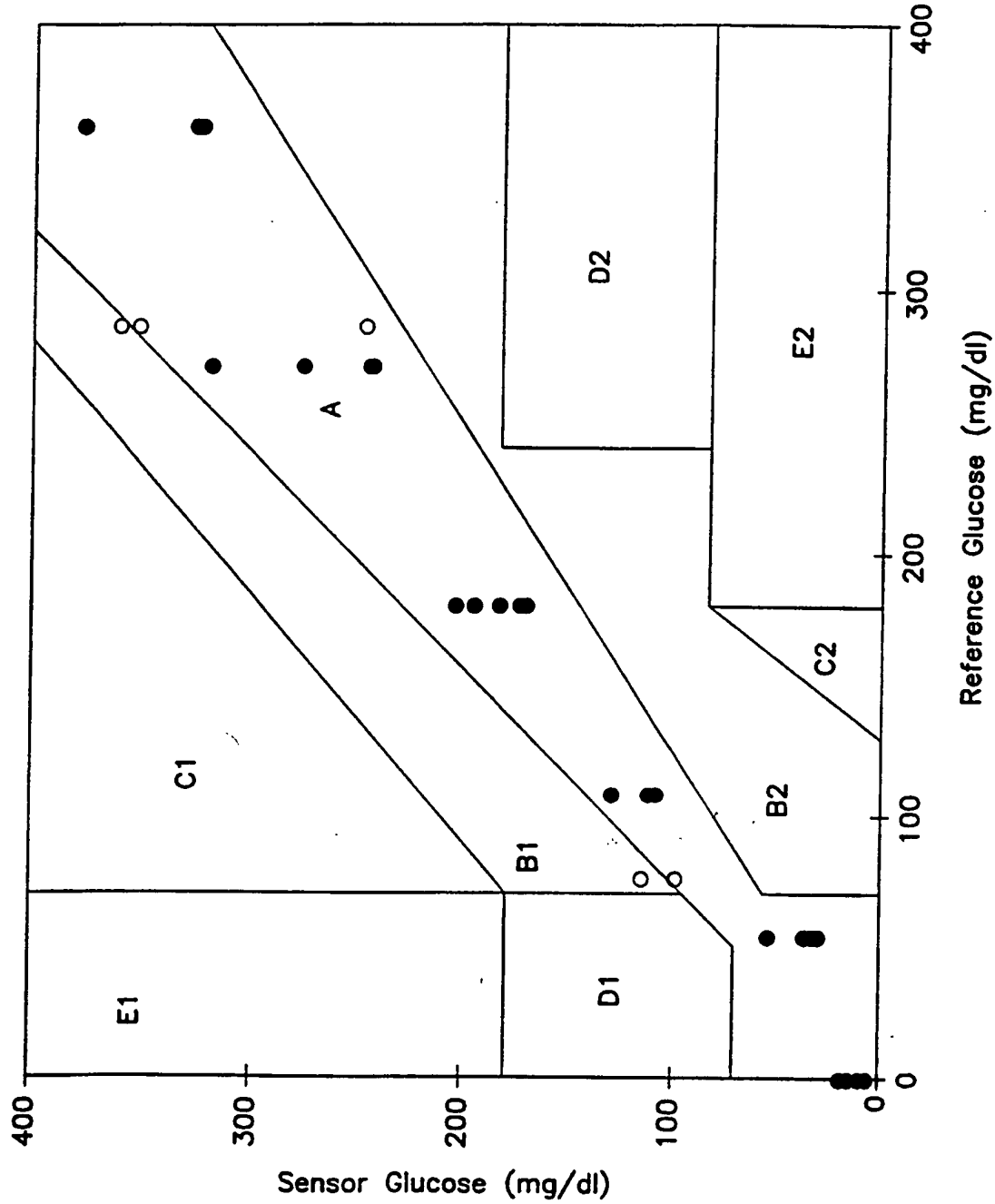

A
B1
B2
C1
C2
D1
D2
E1
E2
400
300
200
100
0
Sensor Glucose (mg/dl)
Reference Glucose (mg/dl)

FIG. 9

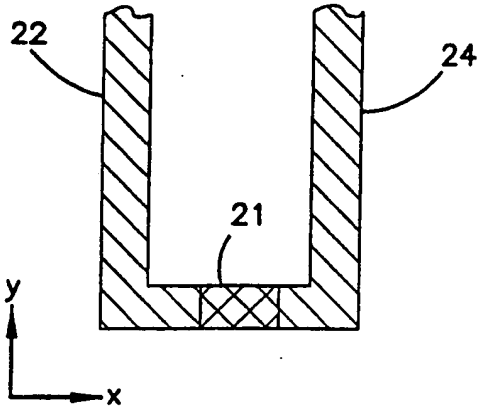


FIG. 11A

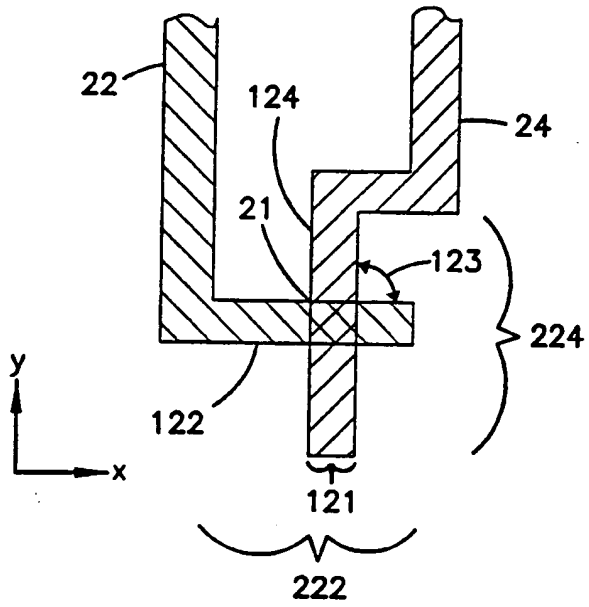


FIG. 11B

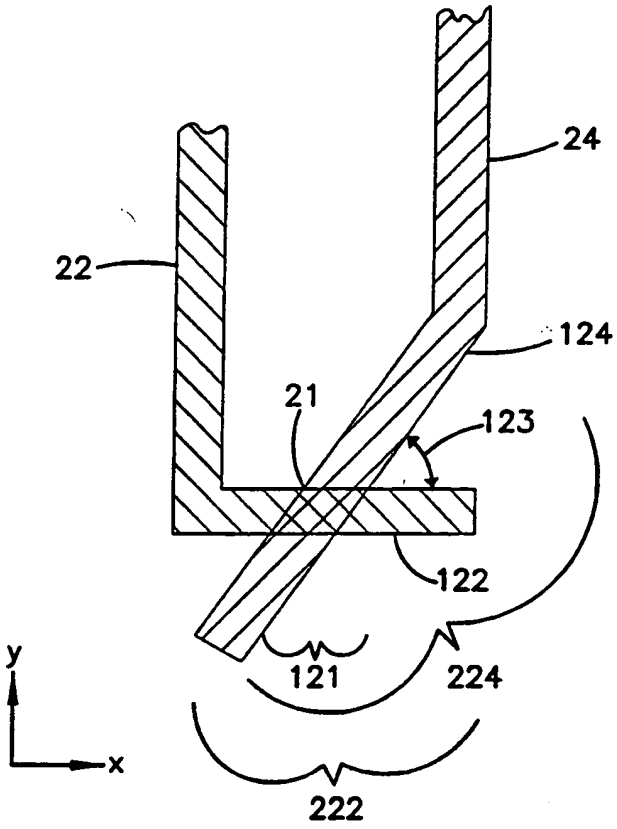


FIG. 11C

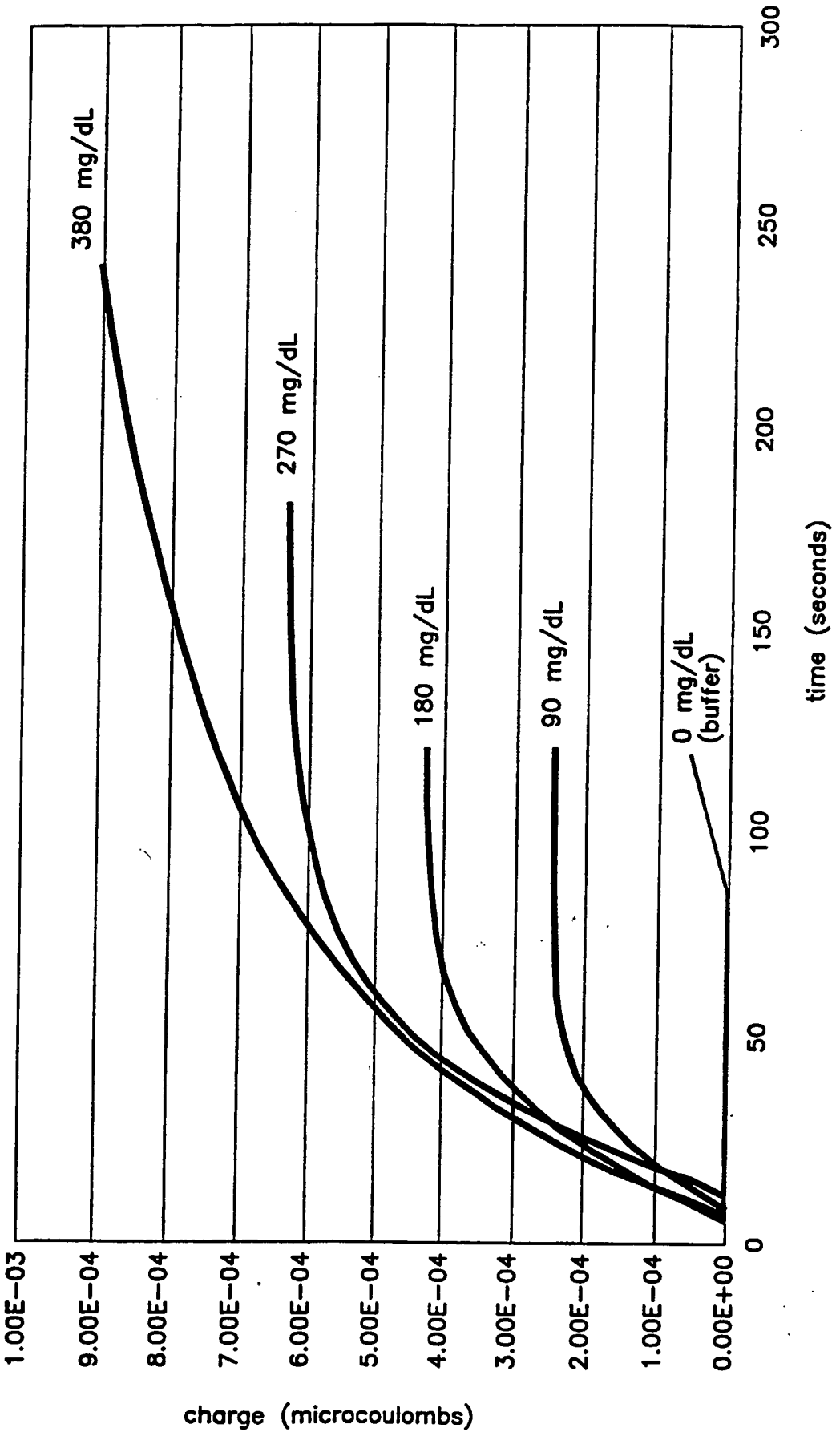

380 mg/dL
270 mg/dL
180 mg/dL
90 mg/dL
0 mg/dL (buffer)
1.00E-03
9.00E-04
8.00E-04
7.00E-04
6.00E-04
5.00E-04
4.00E-04
3.00E-04
2.00E-04
1.00E-04
0.00E+00
charge (microcoulombs)
0
50
100
150
200
250
300
time (seconds)

FIG. 15

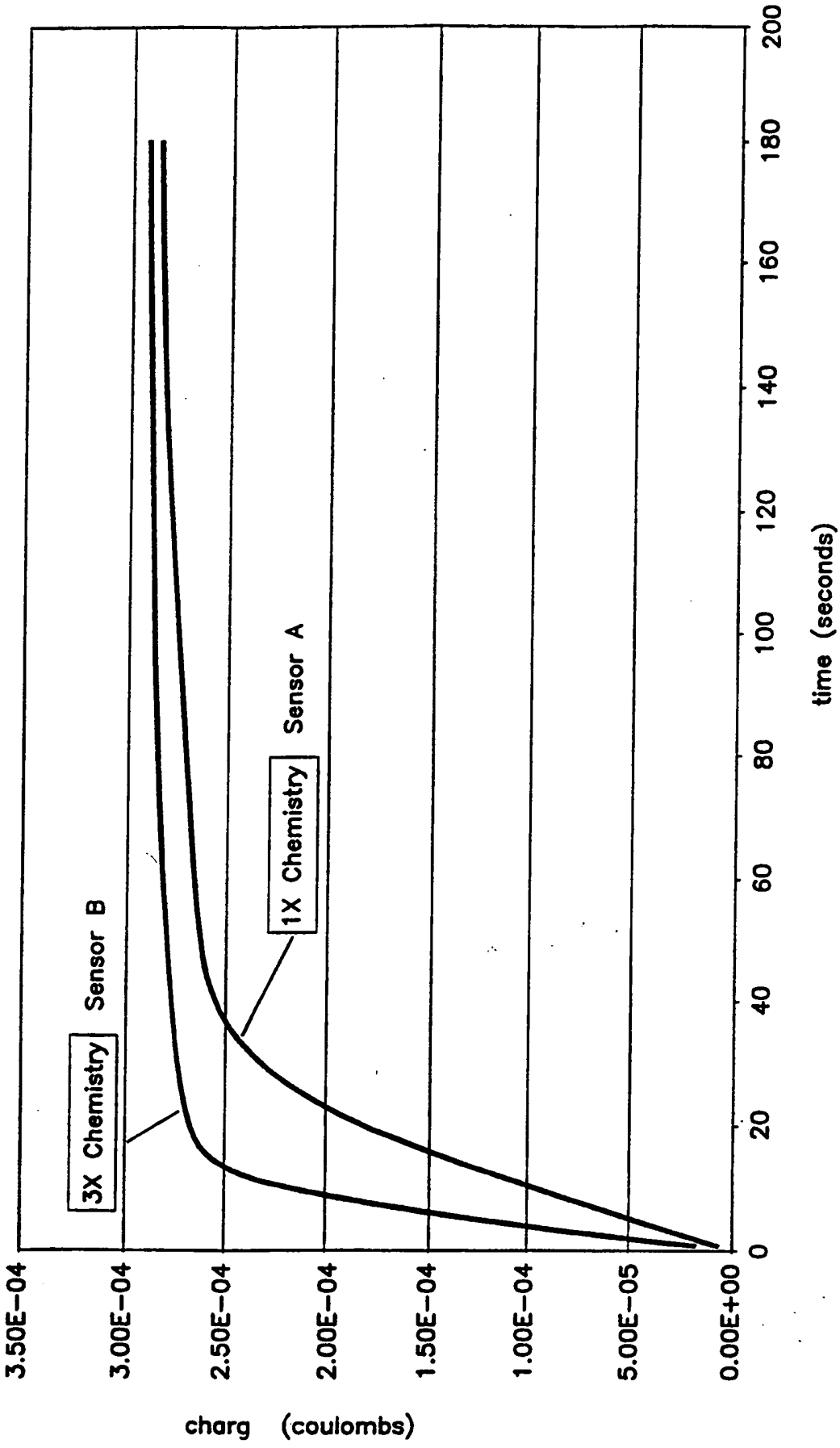

3X Chemistry
Sensor B
1X Chemistry
Sensor A
3.50E-04
3.00E-04
2.50E-04
2.00E-04
1.50E-04
1.00E-04
5.00E-05
0.00E+00
charg (coulombs)
0
20
40
60
80
100
120
140
160
180
200
time (seconds)

FIG. 17

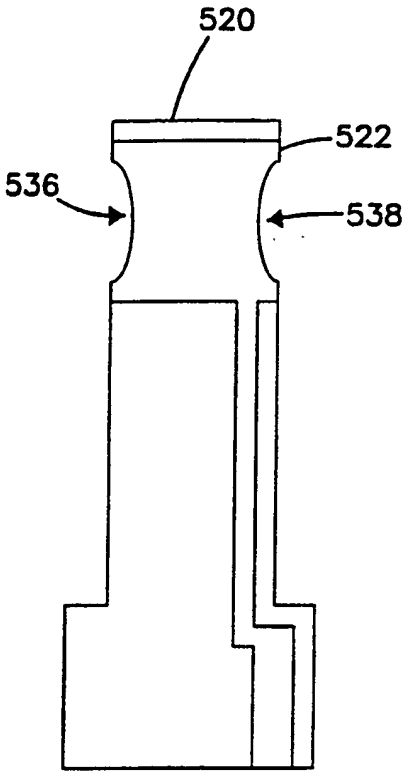


FIG. 19A

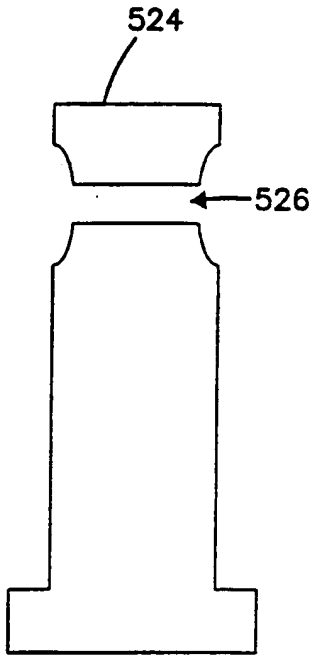


FIG. 19B

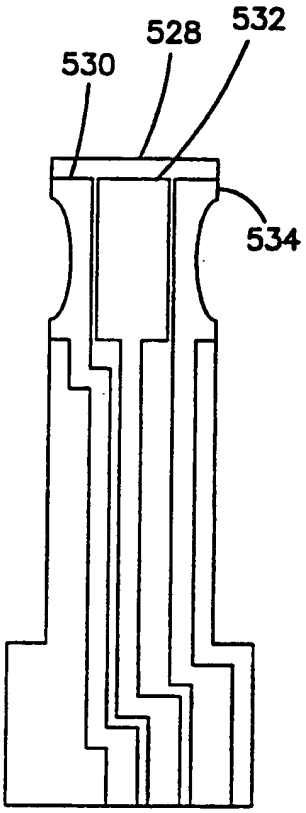


FIG. 19C

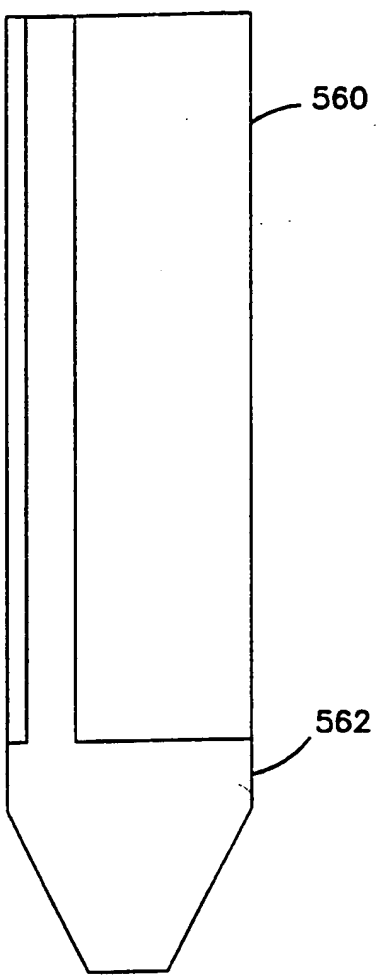


FIG. 21A

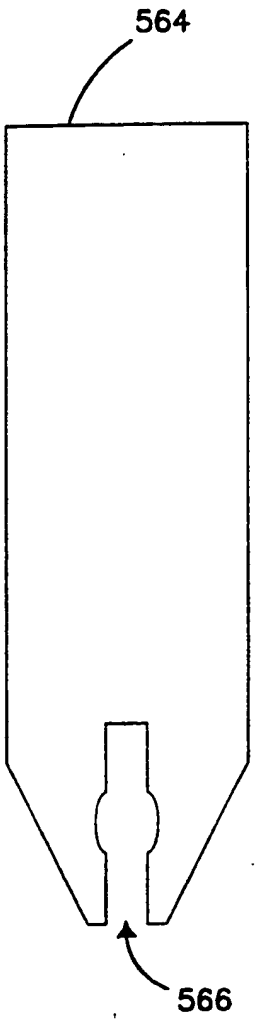


FIG. 21B

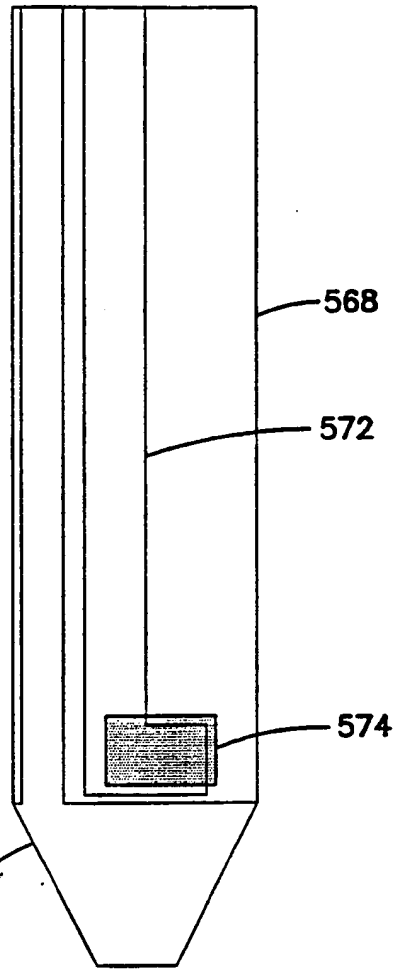


FIG. 21C

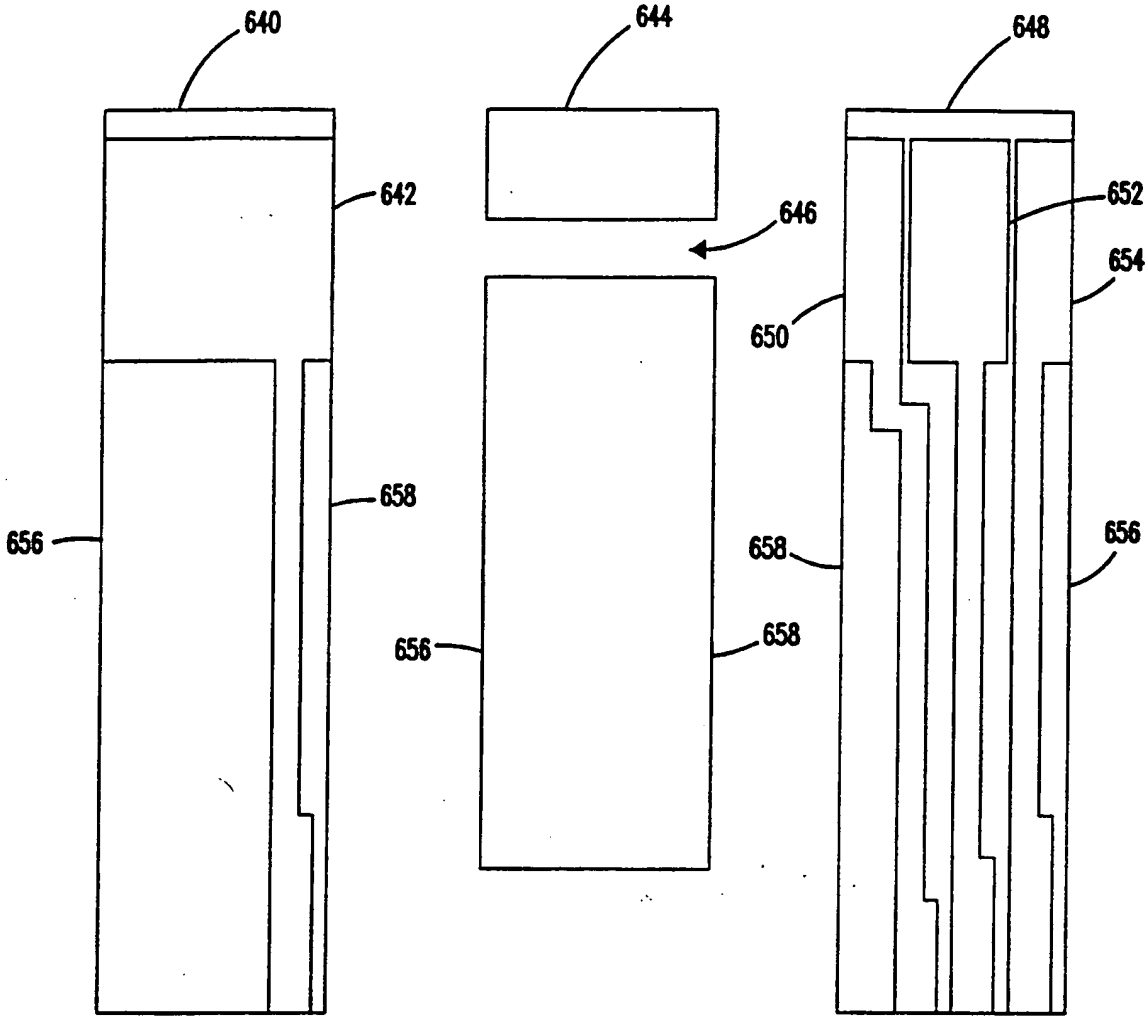


FIG. 23A FIG. 23B FIG. 23C

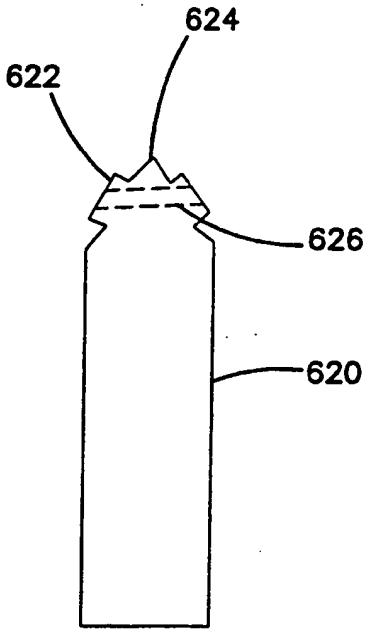


FIG. 25

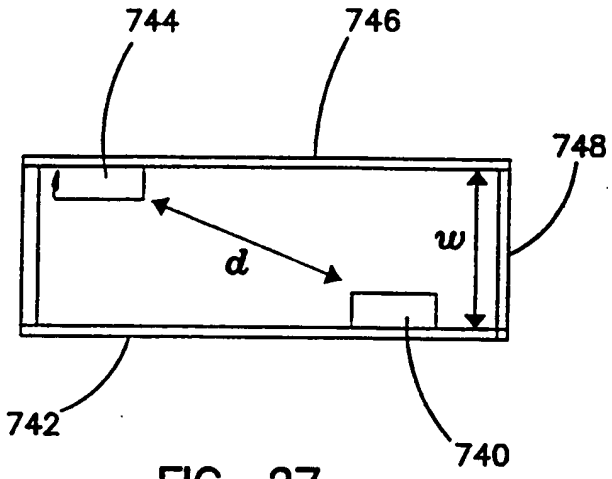


FIG. 27

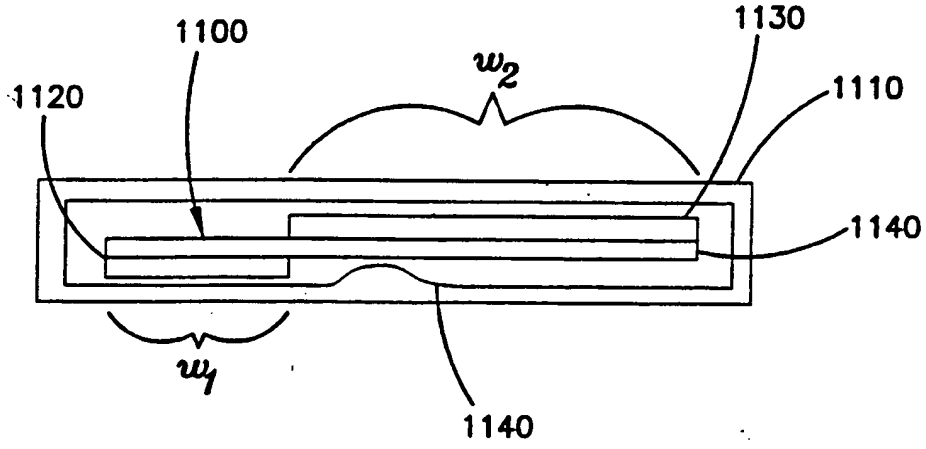


FIG. 32

FIG. 28

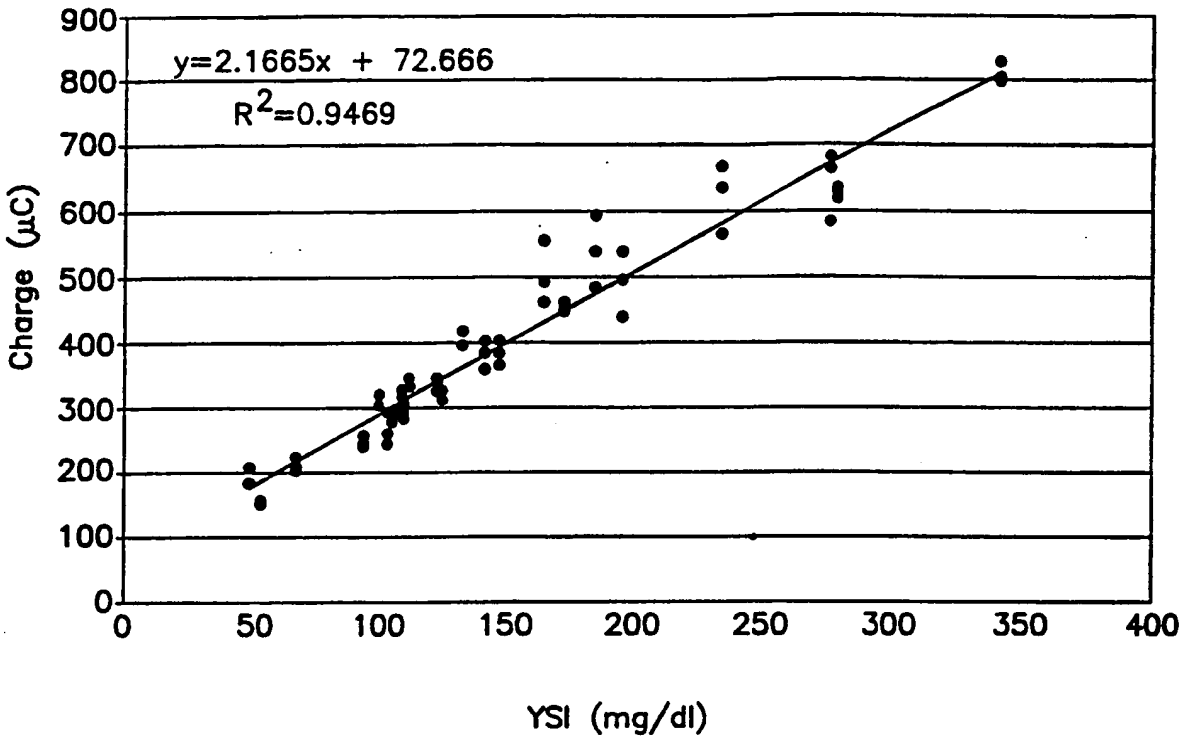
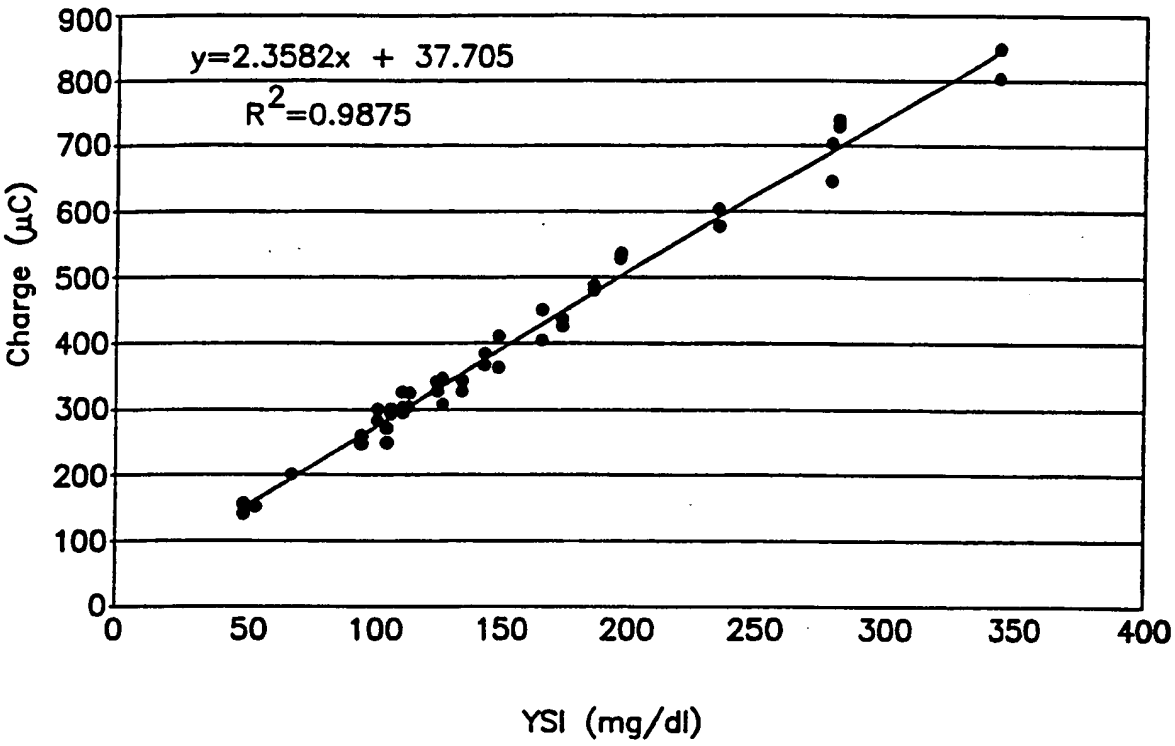

y=2.1665x + 72.666
R2=0.9469
Charge (µC)
YSI (mg/dl)
0
100
200
300
400
500
600
700
800
900
0
50
100
150
200
250
300
350
400

FIG. 30


y=2.3582x + 37.705
R2=0.9875
Charge (µC)
YSI (mg/dl)
0
100
200
300
400
500
600
700
800
900
0
50
100
150
200
250
300
350
400

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/23425

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 98 35225 A (TOMASCO MICHAEL F ;HELLER ADAM (US); HELLER E & CO (US); SAY JAMES) 13 August 1998 (1998-08-13) the whole document	1,2,4, 30,35, 40,41
Y	VIDAL J C ET AL: "A chronoamperometric sensor for hydrogen peroxide based on electron transfer between immobilized horseradish peroxidase on a glassy carbon electrode and a diffusing ferrocene mediator" SENSORS AND ACTUATORS B,CH,ELSEVIER SEQUOIA S.A., LAUSANNE, vol. 21, no. 2, 1 August 1994 (1994-08-01), pages 135-141, XP004012315 ISSN: 0925-4005 abstract	1,2,4, 30,35, 40,41

-/—

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 February 2000	18/02/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Duchatellier, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Ir. ational Application No
PCT/US 99/23425

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		DE 69020908 T	15-02-1996
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		EP 0873514 A	28-10-1998
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US 5695947 A	09-12-1997	NONE	